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VOLUME II JULY, 1915 NOS. 2 AND 3

THE SOUTH AFRICAN MULBERRY BLIGHT *BACTERIUM MORI* (BOY. AND LAMB.) SMITH.

BY ETHEL M. DOIDGE, M.A., F.L.S.

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(With Plates XIX–XXIV.)

FOR some years it has been evident that the finer varieties of the mulberry, particularly the one known as the "English mulberry," do not thrive in certain districts of the Union. I have seen trees of this variety 14 years old which have only attained to a height of three to five feet. In other districts the black mulberry grows well, and fine trees may be seen, so that the failure cannot be attributed to unsuitability of climate; the readiness with which the common mulberry grows would also go to disprove such a supposition. The general appearance of the trees suggests that they are suffering from some blight: a number of the tips of the branches are dead, and the leaves towards the end of the season become covered with small brown spots.

In November, 1908, leaves and twigs so affected were sent in for examination from a farm in the Pretoria district and the diseased areas were found to be occupied by countless swarms of bacteria. No fungus was found in connection with the disease, so that the bacillus was probably the causal organism, and culture work was undertaken in order to discover whether or not the South African mulberry blight is identical with that known in Europe and America.

The literature on the mulberry blight shows that the disease has been attributed to two entirely different bacilli, and it therefore becomes necessary to briefly review such literature in order to compare the characters ascribed to these organisms.

Literature.

In Italy the mulberry blight has received considerable attention owing to its supposed connection with the disease of silkworms known as "flâcherie." The work of the Italian writers will be considered first.

The blight on the leaves was first studied in 1890 by Cuboni and Garbini (2), who attributed the trouble to a Diplococcus. They claimed to have isolated the organism in pure culture, and with it to have reproduced the disease in healthy trees. They held that this Diplococcus was related to, though not identical with, *Streptococcus bombycis* (Flügge) the causal organism of "flâcherie" and found that it was pathogenic to silkworms.

Macchiati (3, 4, 5) working in the years 1891–2 also found the bacillus parasitic on the mulberry pathogenic to silkworms but quite distinct from *Streptococcus bombycis*. He studied the organism more fully and named it *Bacillus cubonianus*.

Voglino (10) published a paper on the bacteriosis of the mulberry in 1894. He isolated from affected tissues an organism pathogenic to silkworms; however, his description of this organism does not agree with that of *B. cubonianus* and his results have not been confirmed by more recent workers.

The most recent of the Italian writers is Peglion (7), who in 1897 confirmed Macchiati's work and gave an account of the disease and the organism causing it. A brief résumé of this paper follows in order that the characters which he assigns to *B. cubonianus* may be compared with those of *Bacterium mori* to which the disease has been attributed by French and American writers.

The first traces of disease are found on the leaves, a slight discoloration of the parenchyma being noticeable at certain points, especially if the leaf is held up to the light. Many of the spots affect the veins, then the leaves become curled and wrinkled, and in any case they become torn and finally reduced to tatters. The young shoots are also attacked; first of all projecting blisters are formed, bright brown in colour, later they become dull and sunken. The first apical internode, where the tissues are tender, is usually the one attacked. The infected area extends in the form of a streak 3–4 cm. long; if only one side of the shoot is affected it causes curvature, but frequently the affected area extends all round the shoot, in which case the extremity wilts and falls.

From such tissues a yellow bacillus was isolated, which rapidly

liquefies gelatine, and grows well on agar and potato, becoming intensely yellow. It does not exceed 2 μ in length, the average being $1\frac{1}{2}$ μ; chains were observed in gelatine cultures. With cultures of this organism suspended in distilled water, characteristic infections were obtained in three days. The experiments, however, were made with detached leaves and shoots kept moist under a bell jar, and there is no record of any controls having been kept.

A study was also made of the anatomical characters of the diseased tissues. In the shoots the cortical tissues are first attacked; infection then progresses in a radial direction as far as the cambium, and sometimes the wood is affected, the latter only in cases where infection takes place before the tissues are properly differentiated. In the infected tissues the cell contents slowly disintegrate and become transformed into a brown substance which is not stained by ordinary aniline dyes. The death of cells in the infected areas and the continued growth of the surrounding parts lead to the formation of small longitudinal cracks; these are frequently filled up the following year by the formation of scar tissue.

In 1894, Boyer and Lambert (2) in France described a bacterial blight on the leaves and shoots of the mulberry, the external effects of which were very similar to those of the Italian blight. They isolated an organism from the diseased tissues and with cultures of this they reproduced the disease on healthy bushes. They named their organism *Bacterium mori* but did not describe it.

A paper published in *Science* in 1910 gave the results obtained by Erw. F. Smith (8), who studied the disease in America. He was unable to obtain infections with any yellow organisms which liquefied gelatine but isolated a white organism which was markedly pathogenic. He obtained numerous infections with pure cultures of this organism and so also did several of his co-workers, working independently. The organism and its effect on the host plant were described in detail, and the name *Bacterium mori* was retained. As this organism appears to be identical with the one isolated from blighted mulberry shoots in this country, a detailed account of the bacterium is given elsewhere. Smith concluded that either there are two organisms (*Bacillus cubonianus* and *Bacterium mori*) capable of causing a blight of the mulberry, or that the Italian workers secured inoculations with mixed cultures.

In 1914, Smith (9) published a further note stating that he was convinced of the identity of the French and American blights, having seen when in Paris, specimens of blighted mulberry twigs collected in

France. He was also shown a culture of a white organism and twigs and leaves blighted by it, which had been inoculated some six weeks previously. The signs, internal and external micro- and macroscopic, on the tree agreed perfectly with the American disease and the streaks looked exactly the same.

Infected shoots and leaves were sent to Washington; from these *Bacterium mori* was isolated and successful inoculations made. In view of the fact that both American and French diseases are certainly caused by *B. mori*, Smith considers that the Italian disease should be re-examined; all the external signs of the disease being identical in both countries.

Geographical Distribution in South Africa.

The mulberry blight is very severe in the Pretoria district; I have not seen a single black mulberry (*Morus nigra*) which has escaped infection. Not only are trees in the town and its neighbourhood affected but in such farms and outlying places as Garstfontein and Onderstepoort.

It is pretty general in Natal, and specimens have been received from Bloemfontein, O. F. S., and Pietersburg, Transvaal, very badly infected with the blight.

Mr R. A. Davis, the Government Horticulturist, informs me that he has never seen the disease in the Western Province of the Cape, and that there one finds very fine specimens of the black mulberry tree. Possibly the winter rains and dry summers are partly accountable for the immunity of the trees in this region, as the new infections on leaves and shoots are only observable in the Transvaal after the first spring rains.

The common mulberry never becomes conspicuously blighted in nature although it is possible to infect its leaves and shoots with pure cultures of the causal organism.

At Mr Davis's suggestion we obtained specimens of leaves of several varieties of mulberry grown by Mrs Forbes of Athol in the Ermelo district. Of these, *M. nigra* and *M. alba* were found to be infected and also another species which has not been identified. This is the only record of varieties other than *Morus nigra* being found infected with the blight.

External Characteristics of Disease.

The appearance of leaves attacked by the bacterial blight is quite distinct from that of leaves attacked by the common leaf spot fungus (*Septogloeum mori* Bri. and Cav.); the spots being smaller, darker in colour and more numerous.

The first indication of infection is the appearance on the under side of the leaves of very minute water-soaked areas (Plate XIX, fig. B); these increase somewhat in size though individual spots rarely exceed 2 mm. in diameter. After some days the spots begin to discolour, and gradually become dark brown or almost black. They are always angular and often become white in the centre. The attacked tissues become quite dead and later fall away leaving holes in the leaf-tissues, and the leaves assume a torn and ragged appearance (Plate XIX, fig. A); they are sometimes completely reduced to tatters. Frequently a large number of infections on a young leaf occur in the neighbourhood of a vein, in which case the vein is affected and becomes wrinkled up and growth ceases in the affected region, with the result that the leaf is distorted (Plate XX, fig. A). Badly affected leaves sometimes turn yellow and fall.

On the shoots infections are also frequent, and usually take place in rapidly growing tissues. They first appear as short, water-soaked, somewhat raised streaks, which may increase to a length of 3–4 cms. The infected portions later become sunken and discoloured (Plate XXI). If the whole of the circumference is affected the young shoot dies, and trees attacked by blight are readily detected by the numerous bare, dead twigs which they bear (Plate XX, fig. B).

This blighting of the young shoots when they appear in spring is also largely responsible for the stunted appearance of the trees, as it is only when the rains are exceptionally late and the disease consequently slow in spreading that the trees are able to make any appreciable amount of new growth.

The tissues in the affected streaks being killed and the rest of the stem continuing to grow, a tension is set up which results in the formation of longitudinal cracks in the diseased parts (Plate XXI).

Attempts at Control.

All the ordinary fungicides have been tried in order to check this disease, particularly Bordeaux mixture, but this has proved quite useless. Mr F. J. Birkett of Dundee, who has been experimenting with various methods, reports good results by using lime-sulphur as a spray and cutting away a large quantity of diseased wood during the winter. It is too soon to judge whether this treatment has been really effective, but the pruning away of diseased material is certainly a sound step, if the prunings are promptly burnt.

Infection Experiments.

From the first specimens of blighted leaves from the Pretoria district which were seen in November, 1908, a white bacterium was isolated and numerous infections were obtained on two young trees in the greenhouse by spraying them with a suspension of the culture in distilled water. At that time the work was not continued as there was other work on hand of a more pressing nature.

In September, 1913, diseased leaves were received from Pietersburg, and from these a white bacterium was again isolated. On the 25th of that month a pure culture of the organism was suspended in sterile distilled water and sprayed on a young mulberry tree (common variety) with an atomiser. Numerous minute water-soaked spots were visible on the leaves on the 30th; these became larger and by the 9th October were beginning to turn brown. Control trees sprayed with distilled water showed no trace of infection. From spots on this tree the organism was again isolated and with the second series of cultures a second tree was inoculated. The leaves were sprayed as before and the shoots pricked with a fine needle. In five days the characteristic spots developed on the leaves, and on the stems in the neighbourhood of the needle pricks a somewhat raised, water-soaked looking streak which later became swollen and discoloured.

What was apparently the same disease was observed at Bloemfontein, O. F. S., and at Dundee in Natal. Spotted leaves collected at the latter place in May, 1913, and kept dry in the laboratory yielded vigorous cultures in August, 1914. On August 21st, a young mulberry tree which was shooting out vigorously and had a number of young leaves was sprayed with a suspension of a 48 hour old agar culture. The shoots were pricked with a fine needle. Very numerous, minute, water-soaked spots were visible on the under side of the leaves on

August 27th (Plate XIX, fig. B), the young leaves showing by far the greater number. The number of spots varied from 20 to several hundred on each leaf, and many of them were in the neighbourhood of the veins. The spots did not begin to discolour for more than a week. Leaves which had been infected in the neighbourhood of the veins before the leaf had attained its maximum size were wrinkled and distorted and frequently curved over to one side (Plate XX, fig. A). After two months most of the infected areas had dried up and fallen out, leaving the leaves torn and ragged (Plate XIX, fig. A).

Infections were also obtained on the stems as described above, the affected area forming a streak 1–4 cms. long. Numerous small stem infections also occurred in parts of the stem which had not been pricked.

On August 28th the organism was again plated out from one of the recently infected leaves and a pure culture immediately obtained, and with this infections were again obtained on a young tree.

In the above experiments the common variety of the mulberry was used as no trees of the black mulberry (*Morus nigra*) could be obtained in Pretoria which were not blighted. Controls were kept in every case and these remained perfectly healthy.

Morbid Anatomy.

In addition to the fact that infection can take place through an unwounded surface and that infection is first evident on the under side of the leaf where the stomata are situated, the distribution of the bacteria in the leaf tissues also points to the probability of stomatal infection. Unfortunately, up to the present I have been unable to obtain slides showing very early stages of infection but sections through leaves bearing a few small spots still in the water-soaked stage included a large number showing the conditions depicted in Plate XXII. A dense mass of bacteria crowds the intercellular spaces near the stoma and occupies the substomatal cavity: spaces more remote from the stoma are also occupied but the bacteria in these are not so numerous. This infected area, however, was in rather close proximity to a portion of the leaf in a more advanced stage of infection, and therefore did not furnish any conclusive evidence that the bacteria had entered through the stoma as there was a possibility they had travelled through the intercellular spaces from the neighbouring infected tissues. At this stage the bacteria are entirely limited to the intercellular spaces and have not invaded the cells, which are still intact.

Later, the increasing mass of bacteria wedges the cells apart,

plasmolysis takes place and the contents appear as a contracted disintegrated mass in the centre of the cell which stains deeply with carbol fuchsin. The bacteria then invade the cells themselves and complete their destruction. In this way the palisade cells and the parenchyma of the mesophyll are entirely destroyed at the points attacked. The bacteria also enter the vessels of the fibrovascular bundles.

The conditions described above are those found in infected spots on the leaves of *Morus nigra*. In this species the mesophyll is very loose in texture and the intercellular spaces large, so that the bacteria have no difficulty in penetrating these tissues. The leaf of the common variety is much thinner, the tissues more compact and the intercellular spaces correspondingly small. Sections through leaves infected by pure culture in which the water-soaked spots were just visible on the under surface showed a slightly different state of affairs (Plate XXIII). Here the bacteria have multiplied enormously in the substomatal cavity and for some distance have levered away the epidermis from the adjacent cells.

In the stem if infection takes place, as it frequently does near the tip where the tissues are tender, all parts are equally affected; if the whole of the circumference becomes involved the end of the twig dies. Frequently only one side of the stem is attacked and sometimes the infection penetrates to the pith, which shows a yellow discoloration.

In older parts of the stem the bacteria are for the most part restricted to the cortex and to the vessels of the wood. Tyloses are frequent in the latter. A fairly deep crack forms in the middle of the infected area owing to the strain on the dead cells caused by the living parts of the stem which continue to grow. Round this a cork cambium is formed and the dead cells are cut off by a layer of cork.

The dead tissues in stem and leaf assume a bright brown colour and do not stain with ordinary aniline dyes.

Morphology.

The cause of the disease is a long rod with rounded ends, usually occurring singly or in pairs, less frequently in long or short chains. The latter are found in the pseudo-zoogloea formed on the surface of beef-broth and other liquid media.

No spores or capsules were observed. The limits of size were found to be 1·5 to 5 μ by ·8 to 1·2 μ, the majority being from 2·5 to 3·5 μ in length.

The organism is actively mobile in a hanging drop culture made

from an agar streak 1 to 4 days old; the rods occur singly, in pairs or in short chains, and the motion consists of darting or tumbling movements. The single rods do not progress far in one direction but make short, swift darts interrupted by tumbling movements. The chains move forward in a sinuous manner.

Examination with the dark ground illumination shows that the flagella are polar and that they are at the forward end of the rod as it moves.

These flagella stain readily by Ellis's modification of Loeffler's method. In the first preparation the majority of the rods showed two, three and four flagella, and, since Smith (8) describes the organism as possessing one polar flagellum and sometimes two, a large number of slides were prepared from different cultures. In these over 50 % of the rods had four flagella, a large number two or three, and a small minority had one only (Plate XXIV, fig. A). From the cultural characters it seems certain that the organism is the one described as *Bacterium mori* and this is the only morphological difference observed. Possibly when differently treated some of the flagella are dropped, but as Smith does not mention by which method his flagella were stained I was unable to ascertain whether this was the explanation.

The rods stain readily with carbol fuchsin and other aniline dyes but are Gram-negative. Involution forms were observed in broth containing 6 % NaCl.

Cultural Characters, etc.

Colonies on (+ 15) *nutrient agar* are barely visible to the naked eye after 24 hours at 25° C. After 48 hours they are round and white with a smooth margin; the margin subsequently becomes undulate. Surface colonies attain to a diameter of over 1 cm. in thinly sown plates, but the size is much affected by crowding. The internal structure of the colonies is at first homogeneous and later firmly granular. Submerged colonies remain very small and irregular in outline.

Nutrient agar streak. On slant agar (+ 15) there is a fair amount of growth which is smooth, white, flat and spreading with an entire margin. It is translucent, slimy and odourless; the medium is not stained.

Nutrient agar stab. A very thin white line of growth follows the needle track; the best growth is at the top.

Nutrient gelatine colonies (+ 15) are flat, white, slow-growing, more or less round; the margin is at first smooth then undulate-lobulate; no liquefaction.

Nutrient gelatine stab. There is no liquefaction; the best growth is at the top of the gelatine; on the depth of the medium there is no growth, even along the needle track. The medium is not stained and there is no odour.

Potato. On this medium the growth is thin, spreading, glistening, white to dirty white. The medium is slightly greyed; there is hardly any action on the starch.

Nutrient broth (+ 15) rapidly becomes turbid; a pellicle is formed which breaks up and falls to the bottom of the tube, forming a thick, white, flocculent sediment.

Litmus milk becomes blue rather rapidly though not so quickly as with *B. campestre.* There was no coagulation or other change during the two months the tubes were under observation. The reaction is continuously alkaline.

Cohn's solution. No growth.

Fermentation tubes. There were no gas formation and no clouding of the closed arm in beef broth containing 2 % of the following carbohydrates: dextrose, saccharose, lactose, maltose, glycerine and mannite.

Indol. No reaction for indol except a doubtful one in a single tube.

Nitrates. There was no reduction of nitrates in nutrient broth containing potassium nitrate.

Sodium chloride. The organism grew vigorously in nutrient broth containing up to 6·5 % sodium chloride; feebly in broth containing 6·5 % to 7·5 %. Tubes containing higher percentages remained clear.

Chloroform. The organism can grow vigorously and for a long time in broth over chloroform; but in a number of cases when not copiously inoculated it failed to do so.

Temperature. The organism grows well at 25° C. and also at 20° C. It was killed by 10 minutes' exposure in thin glass tubes to a temperature of 50° C. Smith gives a slightly higher death point, 51½° C. Tubes exposed to a temperature of 48° C. rapidly became turbid.

Desiccation. Cultures were obtained from leaves which had been drying in the air of the laboratory for 12 months. The resistance of the organism to drying on cover glasses was not tested.

Sunlight. Thinly sown plates were exposed to bright sunlight bottom upwards on ice. The rods were all killed in those exposed for 30 minutes; there was considerable reduction in the number of colonies in those exposed for 15 minutes, and still more after 25 minutes. The exact percentage was not estimated.

Summary.

1. The black mulberry (*Morus nigra*) is very subject in South Africa to a blight affecting twigs and leaves.

2. The blight is fairly widespread but certain districts, particularly the western part of the Cape Province, are as yet free from it.

3. Spraying with Bordeaux mixture is useless in controlling the disease.

4. The organism causing the blight was isolated and numerous infections obtained with pure cultures.

5. The morphological and cultural characters of the organism correspond with those of *Bacterium mori* which causes the French and American mulberry blight.

6. The bacterium as isolated from leaves of blighted trees in South Africa has one to four polar flagella. This is the only important variation from the organism as described in America. Smith describes it with one, sometimes two, polar flagella.

Botanical Laboratories of the Union of South Africa,
Pretoria.

Acknowledgment.

The investigation which forms the subject of this paper was carried out in the Union Phytopathological Laboratories which are under the direction of Mr I. B. Pole Evans, M.A., B.Sc., F.L.S., Chief of the Division of Botany.

I have to thank Mr R. A. Davis, Chief of the Division of Horticulture, for naming the varieties of *Morus* and for valuable information as to the distribution of the disease.

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EXPLANATION OF PLATES.

PLATE XIX.

Fig. A. Photograph of mulberry leaf two months after inoculation with *Bacterium mori*. The diseased tissues have fallen away, leaving the leaf in a ragged condition.

Fig. B. Leaf five days after inoculation, showing a number of small spots in the water-soaked stage.

PLATE XX.

Fig. A. Leaves two months after inoculation, showing distortion due to the disease.

Fig. B. Twigs of *Morus nigra*; the terminal parts of the branches have been killed by the blight.

PLATE XXI.

Figs. A and B. Two photographs of a twig of the common mulberry, showing a number of dark, sunken spots due to infection with *B. mori*. The larger infection at the base of the twig was the result of a needle prick.

PLATE XXII.

Section through water-soaked spot on leaf of *Morus nigra*. Drawn with Edinger's projection apparatus.

PLATE XXIII.

Section through water-soaked spot on a leaf of the common mulberry, fixed five days after inoculation. Drawn with Edinger's projection apparatus. In Plates XXII and XXIII and XXIV B the dots or small strokes are only intended to represent the position of the bacteria, and in no way indicate the comparative size of the rods.

PLATE XXIV.

Fig. A. Rods from a 24-hour old culture on nutrient agar, treated with Ellis's modification of Loeffler's flagella stain. Drawn with the aid of the camera lucida, a Zeiss 1/12 imm. objective and compensating ocular No. 12.

Fig. B. Bacteria in the intercellular spaces; detail from Plate XXIII.

Fig. A

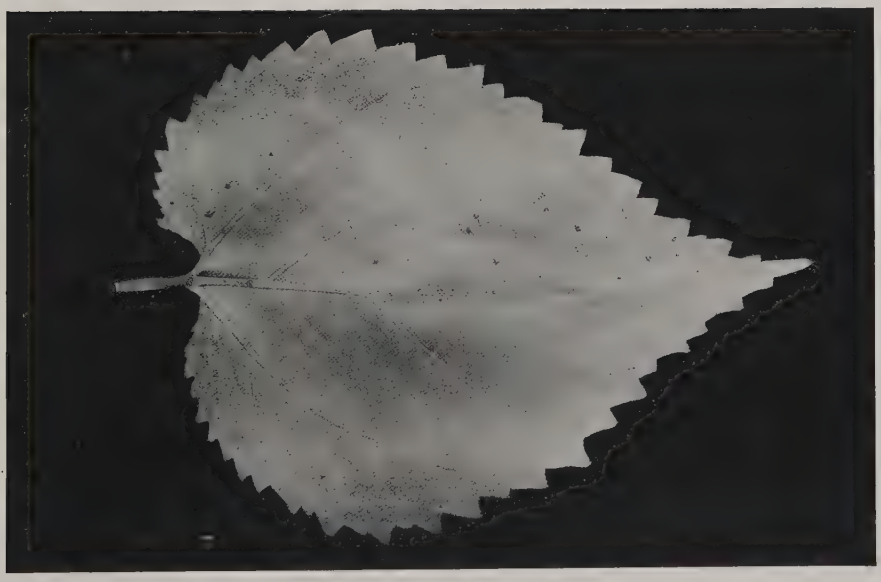


Fig. B

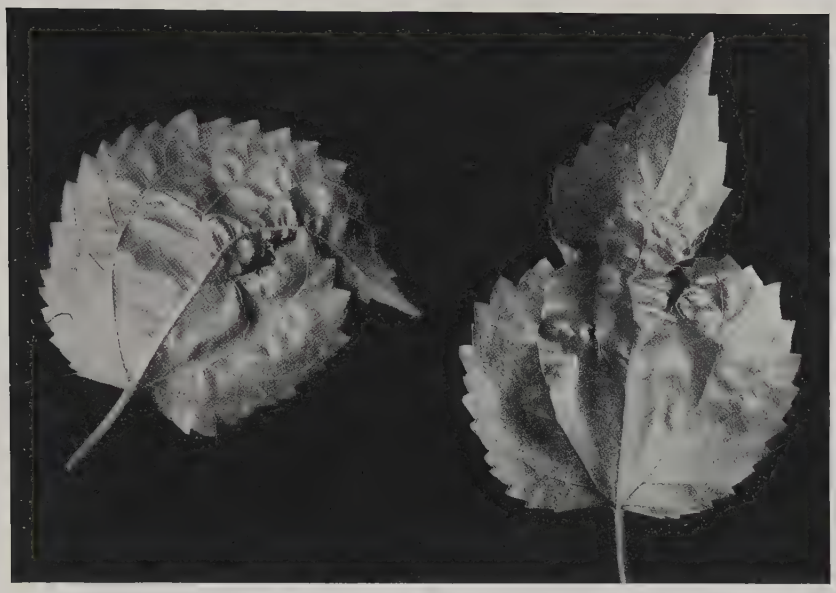
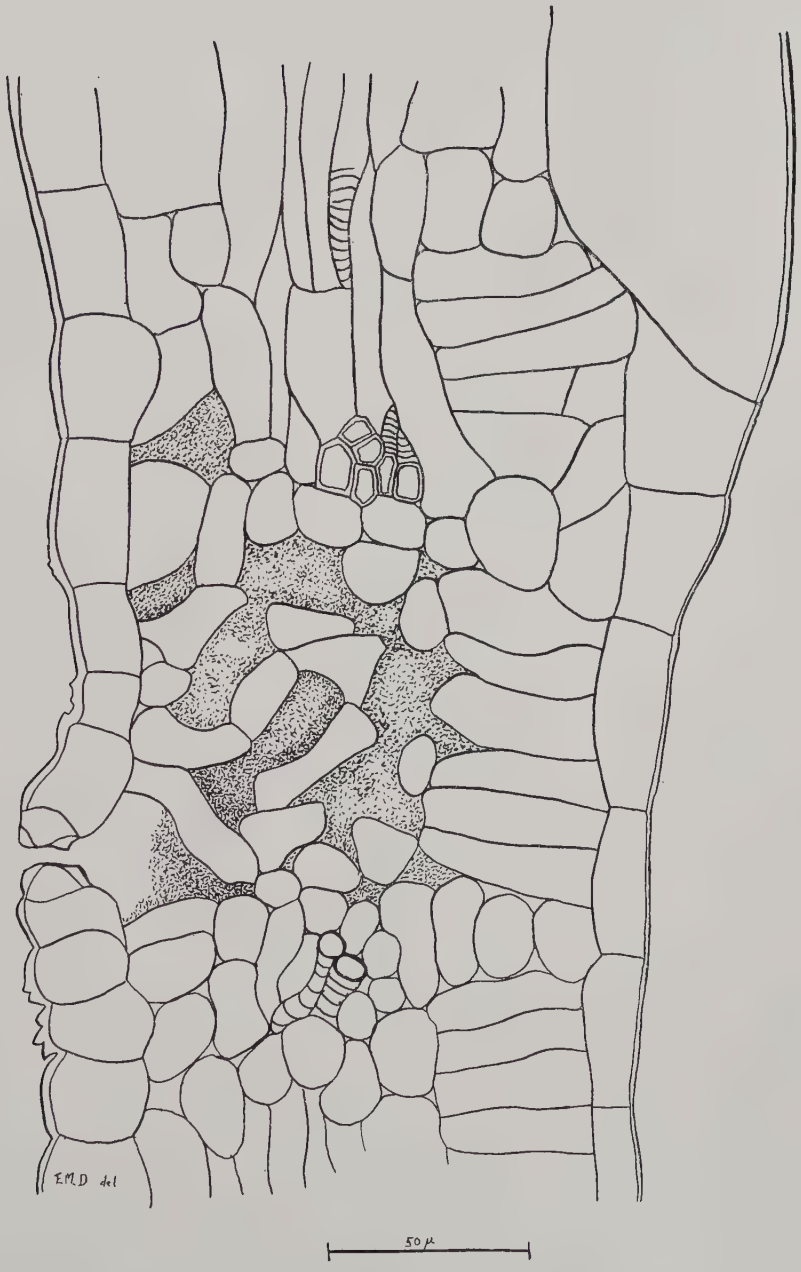


Fig. A

Fig. B

Fig. A

Fig. B


E.M.D del
50 μ


E.M.D. del
50 μ

Fig. A

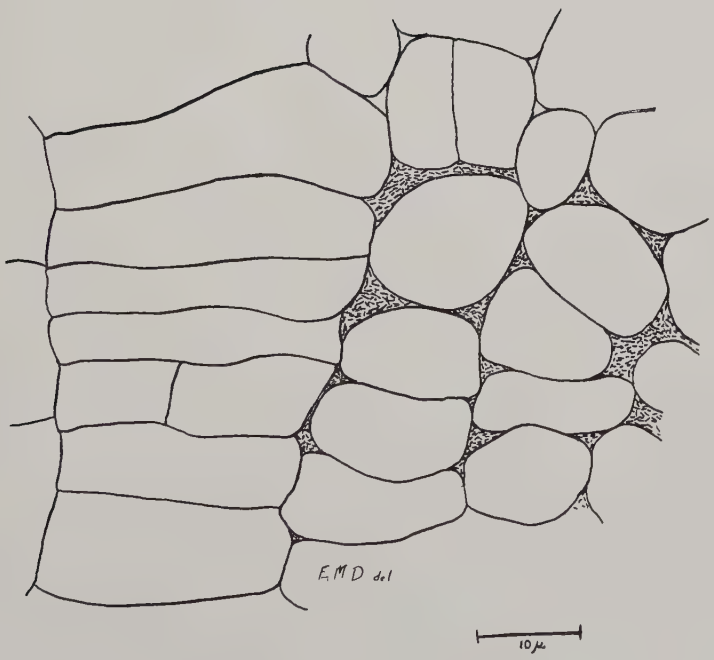


Fig. B

"BLACK NECK" OR WILT DISEASE OF ASTERS.

By WILFRID ROBINSON, M.Sc.

Lecturer in Economic Botany, Manchester University.

(With Plates XXV, XXVI.)

The wilt disease of China Asters is extremely prevalent in market gardens around Manchester, being locally known as "Black Neck" or "Black Leg" disease, and in recent years it has attacked and destroyed large quantities of asters in the district. My attention was drawn to the disease by a large grower of asters at Northenden late in the season of 1913, but the plants then examined were so badly affected that it was impossible to determine accurately the causal organism. Several fungi were isolated from the dead plants but owing to lack of suitable material for inoculation experiments the investigation did not at that time proceed further. Early in 1914, however, the same grower notified me that his seedling asters were seriously diseased, and in the course of a very short time some thousands were destroyed. The seedlings thus attacked formed the starting point for the study of the disease which was under observation in this garden throughout the season.

It soon became clear that the aster may show the characteristic symptoms of the disease at any period in its growth. While many seedlings succumb completely without growing further, the infected plants often continue their growth and may even reach the flowering stage before they collapse. The leaves of such older plants show clear signs of flagging from below upwards, while seedlings exhibiting the same symptoms often droop and damp off. The lower part of the stem shows a very distinct browning or blackening of the tissues for a short distance above the ground level and in many cases the whole of the cortical tissues are decaying. These decaying tissues form an extremely suitable medium for the growth of the various saprophytic fungi, which are usually found in abundance in the latest stages of the

disease. The roots of infected plants are shrivelled and decayed, but infected seedlings often produce new roots so postponing the effects of the attack.

This disease is prevalent wherever asters are grown, but a complete study of it does not appear to have been made. It was first described by Galloway[1] in America in 1896, an undetermined species of *Fusarium* found upon the roots being named as the causal organism; W. G. Smith[2], in this country, at a later date attributed a disease having similar symptoms to a fungus possessing oval spores, which, however, were not figured nor was the species identified. R. E. Smith[3] again in America described the disease, associating it with a fungus which blocks the conducting tissues of the vascular bundles, but as before the fungus was undetermined. More recently Osterwalder[4] has given *Fusarium incarnatum* as the cause of the disease, but having failed to see his paper or an abstract of it I have no knowledge of the evidence upon which his conclusions are based. Massee[5] has described a disease of sweet peas, asters, and other plants which he attributed to *Thielavia basicola*. He found that asters were always killed outright in the seedling stage but no inoculation experiments upon seedlings were described. In the present investigation *Thielavia basicola* has never been observed upon diseased seedlings or older plants.

On the other hand, Friend[6] in 1897 ascribed it to the attacks on the roots by an organism which he named the Aster Worm (*Enchytraeus parvulus*). W. G. Smith[7] also found nematode worms living on the decaying parts of diseased plants. Occasionally I also have observed these worms on diseased roots, but the infection experiments described below indicate that this is not the direct cause of the wilt disease.

Among the saprophytic fungi present on the rotting roots and the lower parts of the stem of badly diseased plants there is usually found a species of *Fusarium*, and this fact seems to have led to its identification as the causal organism of the wilt disease. Up to the present, however, no account of any infection experiments with this fungus on living asters has been given. The results of such experiments described in this paper indicate that while the *Fusarium* may be a secondary or accessory factor, it is not the primary cause of the disease. It has already been

[1] *American Gardener*, vol. 17, p. 518.
[2] *Gardeners' Chronicle*, July 1900, p. 75.
[3] *Bull. Mass. Agric. Coll.*, 1902.
[4] *Landw. Jahrb. Schweiz.*, Bd. 24, 1910, pp. 247–8.
[5] *Kew Bulletin*, 1912.
[6] *Gardeners' Chronicle*, 1897 (August).
[7] *loc. cit.*

mentioned that infected seedlings show "damping off," and this is invariably due to a species of *Phytophthora*, the *Fusarium* not being present in the tissues in the early stages of the disease. This *Phytophthora* always occurs in the tissues of infected asters even when the latter are mature and the characteristic mycelium is easily recognised in sections made near the upper limit of the diseased part of the stem. It will be convenient to describe first the method by which this *Phytophthora* was isolated and grown in pure culture, then to describe its main characters, and finally to give an account of inoculation experiments both with this fungus and the *Fusarium*.

The *Phytophthora* was first detected by the following experiment. A diseased seedling was cut off through the hypocotyl so that a small portion of the infected region was included in the separated part. This seedling was then placed with the cut end in water and examined at the end of 24 hours. In this time an abundant crop of characteristic pear-shaped sporangia had developed from near the cut surface. The similarity of these bodies to the sporangia of certain species of *Pythium* and *Phytophthora* led to the suspicion that one of these Phycomycetes was probably the cause of the disease in asters. It will be seen that all the later work, involving a careful comparison of the morphological characters of the mycelia of the *Phytophthora* and the *Fusarium* as well as experimental infections with both fungi, confirmed the correctness of the above suspicion.

Methods. Observations were carried out on living material from diseased asters of different age, on the fungus grown in pure culture, and also upon carefully fixed material. The last named was prepared by fixing very small pieces of diseased tissue in Flemming's weaker solution, or in chromo-acetic acid weak solution diluted with water to one half strength. These pieces were embedded, cut in serial section, and stained either with Flemming's triple combination or Heidenhain's Iron-alum Haematoxylin followed by Orange G or Bismark Brown. Delafield's Haematoxylin was also found useful for bringing out the cellulose walls of the hyphae in the tissues. Sections from living material were fixed in Iodine and examined in Schultze's Chlor-zinc-iodide solution.

Pure cultures were made on several different media, viz. Aster agar, Beerwort agar, Quaker Oat agar, French Bean agar, Tomato agar, and Salep agar. The aster agar was made by cutting up four healthy, almost full-grown aster plants, and boiling for half an hour in 500 c.c. water. The mixture was then filtered, 10 grams of strip agar added,

and the medium sterilised in the autoclave. The other media were prepared by the ordinary methods given in recent papers by Pethybridge[1], Dastur[2] and others. A more or less healthy growth of the fungus was obtained on all these media but that on Quaker Oat agar was by far the most vigorous. Here an aerial mycelium soon formed a dense woolly felt over the surface of the medium, whilst on other media the growth was much less rapid, aerial hyphae being only sparingly produced.

The fungus was isolated in the following way. A diseased plant was well washed, first in water and then rapidly in a saturated solution of corrosive sublimate. The portion of the stem near the upper limit of infection, as indicated by the discoloration of the tissues, was cut off with a razor previously sterilised. Sections were cut longitudinally from this piece of stem and transferred to the surfaces of Aster agar and Beerwort agar in Petri dishes. After 48 hours the mycelium had spread a considerable distance over the surface of the medium and the growth near its limits was free from bacteria and other fungi. From this region portions of the mycelium were transferred to tubes and plates of sterile media and the cultures in these remained pure. Owing to the very rapid growth on Quaker Oat agar it was necessary on this medium to start fresh sub-cultures fortnightly. New cultures were also started from time to time, by the method described above, from different diseased asters, and in every case the fungus isolated was the *Phytophthora* already referred to.

Although over a hundred artificial cultures have been made up to the present, no mature sporangia have appeared on solid media. The sporangia, however, were obtained in abundance by transferring portions of vigorously growing mycelium to the roots of various seedlings submerged in water in Petri dishes. Among seedlings used successfully for this purpose were those of *Aster*, *Helianthus*, *Gilia*, *Lycopersicum esculentum* and *Senecio vulgaris*. After about three days very abundant crops of sporangia were formed on these pieces of mycelium. The liberation of zoospores was then easily observed by removing the hyphae bearing mature sporangia to hanging drops of fresh well-aerated tap water. It is possible also to obtain sporangia by simply bringing pieces of mycelium from pure cultures into tap water, but under these conditions they do not appear for 16 to 21 days.

[1] *Sci. Proc. Roy. Dublin Soc.*, 1913–14.
[2] *Mem. Dept. Agric.*, India, 1913.

The Mycelium in the Tissues.

The initial infection of aster plants by the *Phytophthora* occurs in the seedling stage through the roots and root-hairs. This was proved by observations on seedlings shortly after infection as well as by the infection experiments which are described below. Many diseased seedlings wilt and succumb almost immediately but others, though infected by the fungus, continue to grow and may even reach the flowering stage before wilting. A comparison of sections of the stem of diseased asters of various ages shows that the wilting, both of seedlings and of older plants, results from the extension of the mycelium to the tissues of the vascular cylinder. The presence of the fungus in the older plants, even though the wilting is delayed, invariably produces serious dwarfing. This effect is obvious in Plate XXV, which is a drawing[1] to scale from a photograph of two plants of the same age, the one attacked by the fungus and the other free from it. Both plants were grown side by side under similar conditions in the same bed.

Transverse and longitudinal sections of diseased plants of different ages show that the mycelium advances upwards in the cortex and for a time at least the cells of these tissues remain living and turgid. The mycelium grows both in the intercellular spaces and through the cells, and in the latter case, when entering or leaving a host cell, a hypha shows a distinct constriction where it passes through the cell wall (Plate XXVI, figs. 1 and 2). Suitably stained preparations show that the hyphae penetrate through the small pits which are frequent in the cell walls (Fig. 3). Haustoria rarely occur, if indeed they are present at all. This is difficult to decide with certainty since branches of the hyphae grow through the cells and the apparent haustoria may simply represent such young branches. Sooner or later the mycelium extends to the vascular bundles. The thin-walled tissues of the phloem especially are attacked and rapidly killed; the hyphae penetrate into the cells of the medullary rays (Fig. 4) and even occasionally send branches through the vessels (Fig. 5). The passages of the last named, however, are not directly clogged by the fungus as was suggested by R. E. Smith[2]; but the proximity of abundant mycelium to the vessels, as well as the drain on the phloem and medullary rays, is sufficient to account for the wilting.

The mycelium consists of sparingly branched hyphae from 3μ to

[1] I am indebted for this drawing to my brother Mr J. B. Robinson.

[2] *Bull. Mass. Agric. Coll.*, 1902.

9μ in diameter, and when young these are non-septate. In later stages and especially in pure cultures septa appear irregularly. The hyphae are multinucleate and have the nuclei disposed at fairly regular intervals (Fig. 2). The structure of the nucleus is somewhat difficult to make out even with the best nuclear stains, but it clearly contains a single nucleolus (Fig. 6). In addition to the nuclei, the vacuolated cytoplasm contains numerous regularly arranged granules which stain readily with nuclear stains (Figs. 6 and 8). Tests with osmic acid and Scharlach R indicated that these granules are not of the nature of fatty bodies. The second of the tests showed fat in the vacuoles of the cytoplasm; the deeply staining granules may therefore correspond to the chondriosomes described and figured by Lewitsky[1] in the young oogonia of *Cystopus Bliti*. Further investigation will be necessary to show whether such an interpretation is correct, but in this connection it is of interest that the granules in question persist in the sporangia and zoospores (Fig. 19).

The walls of the hyphae and also of the sporangia are of pure cellulose and are readily stained violet by Schultze's chlor-zinc-iodide solution. This reaction afforded a ready means of recognising the fungus throughout the investigation, especially when questions of comparison with the *Fusarium* were being considered. The mycelium of the latter stains a deep yellow with this reagent and is therefore readily distinguishable from the *Phytophthora*.

Sporangia.

The sporangia of this fungus have never been observed to be formed except under water; in this respect the species resembles *Phytophthora omnivora* (De Bary[2]) and *Ph. erythroseptica*[3] recently described by Pethybridge as causing the pink rot of potatoes. Prior to the formation of sporangia the vegetative hyphae in the host tissues turn outwards and grow through and between the cells until they reach the epidermis (Fig. 7). They pass through the epidermal cells but the cuticle (*c*) apparently offers some temporary resistance to further progress for each hypha then swells out into one or more rounded branches (Figs. 7 and 8). These gradually separate the cuticle from the epidermis and often form characteristic sorus-like groups (Fig. 7). Each of the

[1] *Berichte der Deutsch. Bot. Ges.* Bd. 31. H. 9, 1913. [2] *Bot. Zeitung*, 1882.

[3] Rotting of potatoes by a new species of *Phytophthora*, *Sci. Proc. Roy. Dublin Soc.*, 1913. Further observations on *Phytophthora erythroseptica*, *Sci. Proc. Roy. Dublin Soc.*, 1914.

swollen bodies is densely filled with protoplasm, contains several nuclei and also large numbers of the deeply staining granules already referred to (Fig. 8). Up to this point, however, no septum has appeared below the swollen portion. The apex of each body becomes very closely pressed against the cuticle and then penetrates the latter by a fine hypha which at once grows out to produce sporangia. The outgrowing hypha may branch at the point of exit (Fig. 9) and produce several sporangiophores or merely give rise to one. Frequently only a single terminal sporangium is borne upon such a sporangiophore, but in many instances series of three or four sporangia arise successively in a sympodial manner. This mode of production of the sporangia is characteristic of several species of *Phytophthora*.

The sporangium arises as a slight swelling of the tip of the hypha bearing it, and this swelling gradually enlarges, becoming first globular and then oval in shape (Fig. 10 *a*, *b*, *c*). At maturity it measures on an average 32μ by 60μ, being then separated from the hypha bearing it by a transverse septum. The apex of the sporangium does not always show a very definite papilla as in other species of *Phytophthora*. As it approaches maturity a large central vacuole appears (Fig. 12) and this corresponds in position and appearance to the central oil body described by Pethybridge[1] in *Ph. erythroseptica*. A little before maturity the vacuole disappears and then the contents of the sporangium are seen to have divided up into 13 to 15 zoospores (Figs. 13, 14, 15). The sporangia germinate while still attached to the stalk and have never been observed to fall off as in *Ph. infestans*. Germination of the sporangia may take place either by the liberation of motile zoospores (Fig. 18) or under different conditions by the direct production of a germ tube (Fig. 17). This corresponds to the observations of De Bary[2] and of more recent investigators on different species of *Phytophthora*.

Reference has already been made to the method by which the liberation of zoospores was observed. The discharge takes place immediately on transferring ripe sporangia into fresh well-aerated tap water. It is brought about by the solution or the opening of the apical portion of the sporangium, and a vesicular swelling-out of the apex has never been observed. The zoospores mature within the sporangium and are directly discharged in a mass, in groups of two or three or one after another. As has been observed by many investigators in other species of *Phytophthora*, some of the zoospores occasionally fail to be discharged

[1] *loc. cit.* [2] *loc. cit.*

but germinate within the sporangium (Fig. 16). In some cases the discharged zoospores at first remain aggregated together and show very sluggish amoeboid movements, altering their shape as they move. Gradually, however, they separate from one another and begin to swim about vigorously as most of the zoospores do from the first. In motion the zoospores continually alter their shape but generally are somewhat kidney-shaped with a median furrow (Fig. 18). They possess two vacuoles which appear to lie one on either side of the furrow, and two unequal cilia springing laterally from the middle of the depression. When moving the zoospore progresses in the direction of its longer axis, one cilium lashing forwards and the other trailing behind. After swimming for about half an hour they come to rest (Fig. 19) and immediately begin to germinate by means of a germ tube which soon branches (Fig. 20). In water alone these germ tubes do not grow much further, but on nutrient media they give rise to a normal mycelium. In hanging drops containing a piece of the root of an aster seedling, the germ tubes almost immediately penetrate the tissues and produce a mycelium within.

After the liberation of the zoospores the hypha forming the stalk of the sporangium grows into the empty sporangium and then forms a new sporangium within the first (Fig. 21). As many as three sporangia are in some instances thus formed within one another (Fig. 22). Variations also occur in which the proliferating hypha grows out of the empty sporangium before forming the new sporangium (Fig. 22). So far as I am aware this proliferation of sporangia has not been previously described for any species of *Phytophthora*, although De Bary[1] describes and figures similar examples in *Pythium proliferum* and *P. megalacanthum*.

Fusarium.

The vegetative characters of the species of *Fusarium* so frequently present on the decaying portions of the stems of diseased asters render this fungus easily distinguishable from the primary cause of the disease. The mycelium branches much more freely than that of the *Phytophthora* (Figs. 23 and 24), it stains yellow with iodine and also with Schultze's solution and its walls give none of the reactions characteristic of cellulose. The hyphae are abundantly septated, with individual cells containing several nuclei and characteristic oil globules, but none of the regularly

[1] *Bot. Zeitung*, 1881, pp. 559–623.

disposed granules that are invariably seen in the *Phytophthora*. Conidia are produced in great abundance, are typically four- or five-celled, curved and slightly pointed at the ends (Fig. 24). Although carefully looked for the mycelium of this fungus has never been observed among the living cells of the host plant. On the other hand it is always possible to find the mycelium of the *Phytophthora* in the higher parts of the diseased regions, that of the *Fusarium* always being confined to the lower decaying parts of the stem and to the roots. This *Fusarium* was isolated and grown in pure culture on Beerwort and Quaker Oat agars.

Experimental Infections.

A number of infection experiments was carried out on seedling asters. As a preliminary test, from a number of seedlings growing together in a pot one was selected and a small quantity of mycelium of the *Phytophthora* from a pure culture of the fungus was placed near the collar. After five days the seedling had completely collapsed showing the ordinary symptoms of "damping off." All the other seedlings in the pot remained unaffected for at least 10 days after the experiment. On cutting off the collapsed seedling and placing it in water, hyphae grew out of the tissues and produced typical sporangia in 24 hours.

For more critical experiments a number of aster seedlings were carefully uprooted, washed free from soil and laid in Petri dishes with the roots in water. Three of these were then inoculated by placing on each of the roots a piece of mycelium from a pure culture on Quaker Oat agar. Controls were placed in Petri dishes with the roots in water without being inoculated. Zoosporangia were abundantly produced on the mycelium and the seedlings became infected, the hyphae having travelled one inch in the tissues of the hypocotyl at the end of nine days. The tissues were browned and the seedlings were beginning to collapse. The controls appeared quite normal at the end of the same period and no fungus was present in the tissues.

An exactly parallel series of tests with controls was carried out on seedlings of asters, spores from a pure culture of *Fusarium* (previously isolated from decaying roots of diseased asters) being used for inoculation. After nine days no change was observable and up to the 19th day, when the tests were stopped owing to the complete collapse of the seedlings in the *Phytophthora* inoculations, the *Fusarium* series remained healthy and normal.

Further series of tests were carried out by inoculating seedlings

grown on moist cotton wool in test tubes, with mycelium of the *Phytophthora* and spores of the *Fusarium* as before. Here also the seedlings inoculated with *Phytophthora* collapsed after 9 to 12 days, while those inoculated with the *Fusarium* and also the controls remained unaffected in any way. The Petri dish inoculations were repeated several times and it was invariably found that the seedlings inoculated with the *Phytophthora* showed the characteristic symptoms of the disease under investigation. Sections of such seedlings always showed that the collapse was due to the presence of the mycelium of that fungus in the tissues. The *Fusarium* on the other hand never produced such effects, in fact it was only possible to grow this fungus on decaying seedlings or on those previously wounded.

The stems of almost mature aster plants were also inoculated with mycelium of the *Phytophthora*, a slight wound being made with a sterile scalpel and a piece of mycelium from a pure culture inserted. Controls were also wounded but not inoculated. After 10 days the mycelium had progressed over one inch upwards in the tissues of the stem and had produced the dark discoloration characteristic of the disease. On cutting off one of these stems through the discoloured region, well above the inoculation wound, and placing it in water, the typical sporangia were obtained in 24 hours. It was not found possible to infect older asters without previously wounding the tissues.

Conclusion.

No sexual organs of any kind have yet been found either on the fungus grown in pure culture or upon asters at any stage of the disease. All attempts to produce the oogonia or antheridia in pure cultures by the methods successfully used by Clinton[1], Pethybridge[2], Dastur[3], Klebahn[4] and others for various species of *Phytophthora* have so far proved unsuccessful. The characters already described appear sufficiently striking, however, to warrant a discussion of the systematic position of the fungus to which I attribute the disease of asters described above. Reference has already been made to the close resemblance of the vegetative mycelium and sporangia to those of *Pythium* and *Phytophthora*. A close examination of the characters of these two genera reveals the fact that the main points of difference lie in the modes of formation and liberation of the zoospores. In *Pythium* the

[1] *Rep. Conn. Agric. Exp. Sta.*, 1911. [2] *loc. cit.*
[3] *Mem. Dep. Agric.*, India, 1913, 4.
[4] *Krankheiten des Flieders*, 1909.

sporangium swells out to form a large apical vesicle into which the undifferentiated contents of the sporangium pass. The zoospores then become defined and are liberated by the rupture of the vesicle. In *Phytophthora*, however, no vesicle is formed and the zoospores round off within the sporangium some time before their discharge which takes place directly by the solution or rupture of the apex. In these details therefore the fungus under consideration corresponds to *Phytophthora* and the very large size of the sporangium approximates it to *Phytophthora omnivora* (De Bary). *Ph. omnivora* was described by De Bary attacking a large variety of plants including *Clarkia*, *Gilia*, *Cleome*, *Schizanthus*, *Fagopyrum*, *Oenothera*, and *Epilobium*. A number of other investigators have more recently described fungi morphologically almost identical with *Ph. omnivora*, but owing to differences in the range of hosts which they will attack these have been separated off as distinct species. Examples of species founded mainly upon this physiological distinction are *Ph. cactorum* (Schenk), *Ph. Fagi* (Hartig), *Ph. omnivora* var. *arecae* (Coleman), *Ph. Faberi*, and *Ph. Syringae* (Klebahn). The characters of these various species and their affinities are so completely discussed in recent papers by Pethybridge[1], Dastur[2] and Butler[3] that a further recapitulation is unnecessary here. In this connection, however, it is of interest that I have already found it possible to infect and to obtain sporangia on seedlings of *Gilia tricolor*, *Ricinus*, and *Helianthus annuus* by inoculation with mycelium from pure cultures. Inoculations upon seedlings of *Lepidium*, *Lycopersicum esculentum* and young plants of *Solanum tuberosum* gave negative results in all cases. The failure to obtain oospores in cultures or even in infected seedlings of *Gilia* (in which De Bary found those of *Ph. omnivora* in abundance) indicates that the aster *Phytophthora* is not identical with *Ph. omnivora*, yet it seems likely that it is a physiological form of it. Further research will show whether the sexual organs are of the character described by De Bary for *Ph. omnivora* and whether the range of host plants is as wide as in the case of some of the above species. For the present, therefore, the question of the identity of the species of *Phytophthora* here described for the first time as producing the wilt disease of asters is deferred; but this investigation establishes the fact that the causal organism of the disease is a species of *Phytophthora* and not a *Fusarium* as has generally been supposed. It is also clear

[1] *Sci. Proc. Roy. Dublin Society*, 1913, 1914. *loc. cit.*

[2] On *Phytophthora parasitica* nov. spec., *Mem. Dep. Agric.*, India, 1913.

[3] Studies in Peronsporaceae *Mem. Dep. Agric.*, India, May 1913.

that, though the initial infection occurs in the seedling stage, the disease may not be seriously manifest until late in the life of the plant. In this respect, therefore, the aster disease differs from other diseases caused by species of *Phytophthora* where the destruction of the host plant is very rapidly accomplished.

In conclusion I should like to express my indebtedness to Professor W. H. Lang for his advice and helpful criticism during the course of this investigation, which has been carried out in the Cryptogamic Botany Research Laboratory of the University of Manchester.

Summary.

1. The tissues of asters attacked by the wilt disease always contain the mycelium of a species of *Phytophthora*; this fungus was isolated and grown in pure culture on various media.

2. Several saprophytic fungi including a species of *Fusarium* were isolated from the decaying roots of badly diseased asters but none of these is the primary cause of the disease.

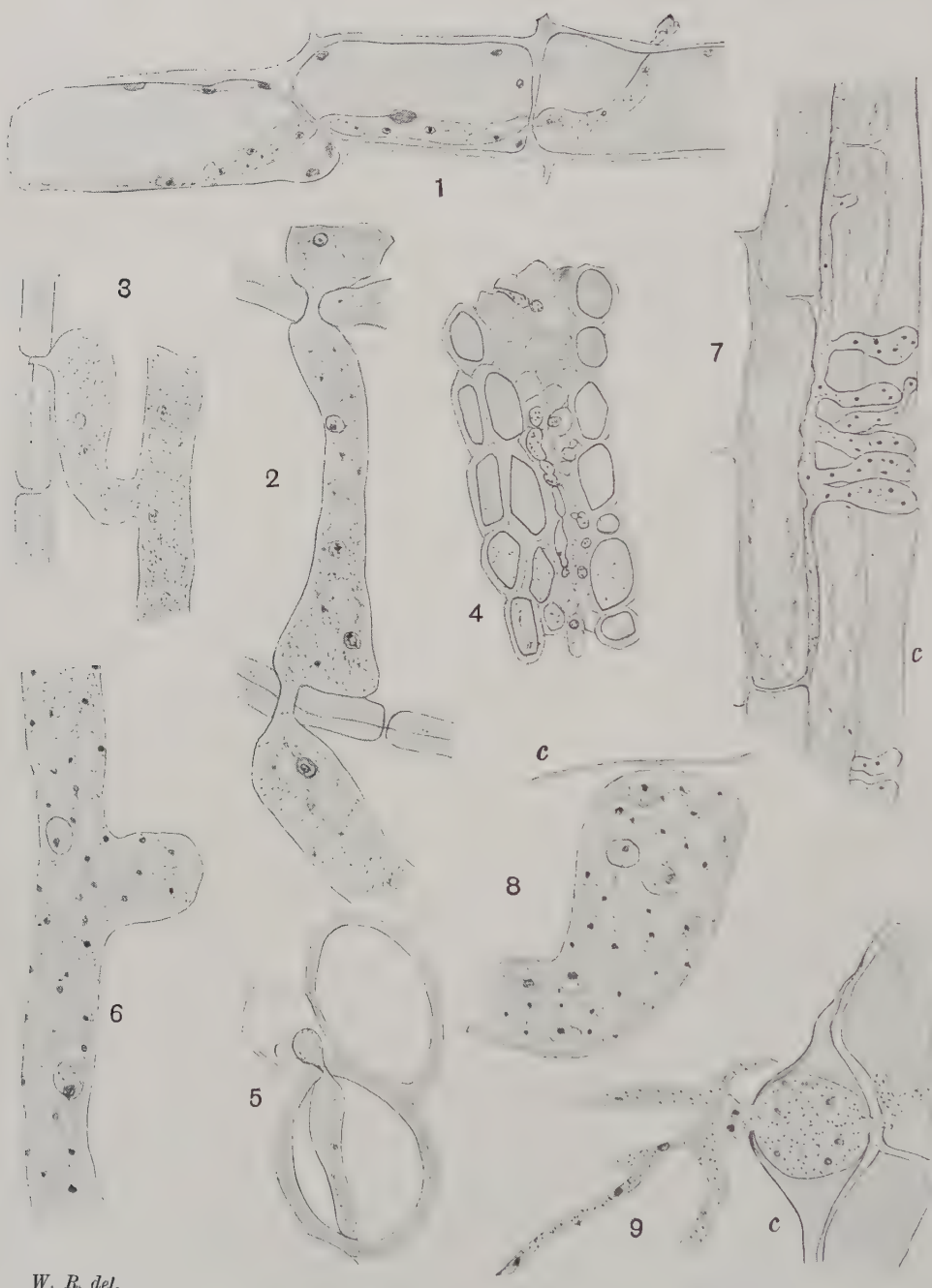
3. A series of inoculation experiments with adequate controls showed that the *Phytophthora* could produce a disease on seedling and mature asters identical in every respect with the "Black neck" or wilt disease.

4. A similar series of inoculation experiments with the *Fusarium* gave negative results.

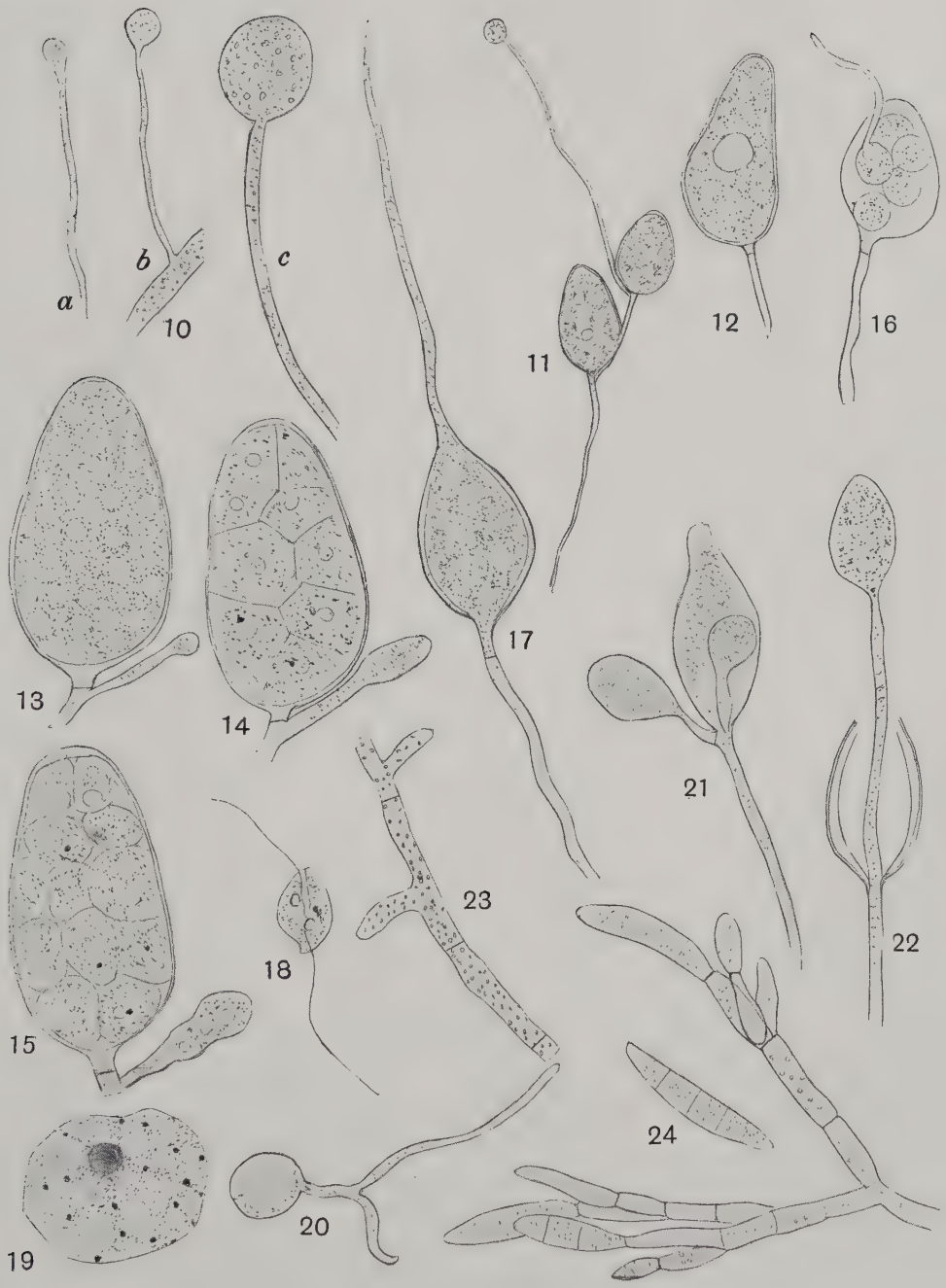
5. The characters of the vegetative mycelium and its relations to the tissues of the host plant were studied in some detail.

6. The sporangia show most of the characters described by De Bary for *Phytophthora omnivora*, but after the discharge of zoospores the stalk of the sporangium grows through and produces a second and even a third sporangium within the first. This proliferation has not, as far as I know, been previously described for any species of *Phytophthora*.

7. No sexual organs have as yet been observed either on infected plants or in pure culture on suitable media.



W. R. del.



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DESCRIPTION OF PLATES.

PLATE XXV.

Two almost mature aster plants from the same bed—the one diseased and the other healthy—drawn from a photograph to scale.

PLATE XXVI.

Fig. 1. Cortical cells of the hypocotyl of seedling aster in *L.S.* showing hypha of *Phytophthora* growing through three cells. The hypha enters and grows through the protoplasm into the vacuole. ×180.

Fig. 2. Intracellular hypha passing through two parallel walls of a cell showing constrictions. ×1440.

Fig. 3. Similar hypha to that in Fig. 2 with tip of branch partially through a pit in the wall of a host cell. ×1440.

Fig. 4. Hyphae entering and growing through the parenchymatous cells of a medullary ray. ×180.

Fig. 5. Hypha entering one of the large vessels of the xylem seen in *T.S.* ×700.

Fig. 6. An intercellular hypha showing nuclei, vacuolated protoplasm and deeply staining granules at the junctions of the meshes of the cytoplasmic network. ×2000.

Fig. 7. *L.S.* of the outer portion of the stem of a diseased aster showing swollen hyphae separating the cuticle (*c*) from the epidermis, prior to the formation of sporangia on the exterior. One of the hyphae has penetrated the cuticle by a fine pore. ×180.

Fig. 8. One of the swollen hyphae similar to those in Fig. 7 showing several large nuclei and the deeply staining granules as in Fig. 6. *c.*=cuticle. ×2000.

Fig. 9. A later stage of a similar hypha to that in the previous figure showing branching as it leaves the cuticle. ×1440.

Fig. 10, *a*, *b* and *c*. Various stages in the formation of the sporangium. ×700.

Fig. 11. Sporangiophore bearing three sporangia in different stages showing sympodial development. ×570.

Fig. 12. Almost mature sporangium showing large central vacuole. ×570.

Fig. 13. Mature sporangium in which division into zoospores is beginning. ×700.

Fig. 14. The same sporangium as Fig. 13 ten minutes later. ×700.

Fig. 15. Same sporangium as in Figs. 13 and 14 drawn 15 minutes later. The zoospores have contracted somewhat from the wall and are more rounded. ×700.

Fig. 16. Sporangium in which some of the zoospores have failed to be discharged—one is seen germinating *in situ*. ×570.

Fig. 17. Sporangium germinating conidially by putting out a hypha. ×570.

Fig. 18. Zoospore as seen in motion showing unequal cilia and two vacuoles. ×700.

Fig. 19. Zoospore at rest stained to show nucleus cytoplasm and protoplasmic granules. ×2000.

Fig. 20. Germinating zoospore. ×700.

Fig. 21. Proliferating sporangium showing the stalk growing into the empty sporangium to form a second. ×570.

Fig. 22. Similar proliferating sporangium. There are seen the empty walls of two older sporangia which have discharged their zoospores. ×570.

Fig. 23. Portion of a hypha of *Fusarium*. ×700.

Fig. 24. Mycelium of *Fusarium* bearing conidia. ×700.

Figs. 10 to 18 and 20 to 24 on Plate XXVI were drawn from material in the living unstained condition.

A CONTRIBUTION TO OUR KNOWLEDGE OF SILVER-LEAF DISEASE.

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The etiology of Silver-leaf disease has passed through many phases and has been the subject of many diverse opinions. First observed by Prillieux (1885), this disease was placed by Sorauer (17, p. 285, "Milchglanz") among the non-parasitic plant diseases. Similarly Delacroix (2, p. 227) places "le plomb" among the "maladies non-parasitaires." Percival (12), Güssow (3) and Brooks (1) record successful inoculation experiments with the fungus (*Stereum purpureum*). Massee (9) rejects this view of the etiology by *Stereum*; Blackmore ascribes the disease to the action of bacteria. Though the view generally adopted seems to be that of Percival, nevertheless it must be admitted that this remarkable phenomenon in phytopathology is not yet completely understood. Such being the case it seems that the methods of practical treatment advocated by various authors have hardly an adequate scientific foundation.

The external symptoms of Silver-leaf disease are obvious. As is well known, the leaves of the attacked trees show on their upper surfaces an ashen-grey colour giving a "silvered" appearance to the tree. The list of host trees is considerable including in particular nearly all the species of *Prunus*. The most important works on Silver-leaf disease are those of Percival (12), Güssow (3), Brooks (1) and Pickering. A complete bibliography and history of the whole question will be found in Güssow's work.

My contribution concerns the cytology of the attacked leaves. For the investigation I made use of the attacked and healthy plum leaves of the variety *Prunus domestica* var. "Victoria" which is a favourite in the English gardens because of its large and valuable fruit. Hand

sections were made of some of the leaves; others were cut with the microtome.

Fleming's strong solution, which is very satisfactory for cytological work, was used as a fixing agent. In it the small pieces of the attacked and healthy leaves were exhausted by the water-pump and afterwards hardened in alcohol and embedded in paraffin. Many slides were made from material of three different ages, *i.e.* the middle of July, the end of September and the second half of October. By this means I perceived that the observed abnormal structures to be described later are not the consequences of autumn disorganisation; it can also be shown that we are not dealing here with artifacts. The sections were stained by different methods but all gave the same results. Mann's method (18, p. 735, Gram's iodine, eosin, toluidin blue), the inverse method (18, p. 809) by mordanting with potassium antimony tartrate, and the method of methyl-green with potassium hydroxide proved the best. Also Heidenhain's hæmatoxylin, safranin with anilin water, and gentian violet followed by eosin in clove oil were found satisfactory for the work.

I.

The anatomical structure of the attacked leaves differs from that of healthy ones. The thickness of the attacked leaves is somewhat greater than that of the healthy leaves. The turgor of the tissues in the former seems to be increased. Güssow (3, p. 393) states as the most striking anatomical phenomenon exhibited by "silvered" leaves, "dass sich die Zellen äusserst leicht von einander lösen und in dem Wasser des Präparates frei herumschwimmen. Nur mit äusserster Vorsicht gelingt es, kleine intakte Sektionen zu erhalten."

I also perceived that the cells of the spongy parenchyma fall apart very easily so that it is very difficult sometimes to obtain the sections intact. This happens only in the sections of fresh material, those cut from the thoroughly fixed and carefully embedded material were quite intact. And from such microtome sections the anatomical relations of the infected leaves could be observed quite readily.

The mesophyll of the leaves attacked by the Silver-leaf disease is thicker than the mesophyll of the healthy leaves or of the healthy parts of the attacked leaf. There are no striking changes in the length and arrangement of the palisade cells. But we notice in the spongy parenchyma that some cells are stimulated to a more intense growth in length; the intercellular spaces are in this tissue greater than in the healthy

part of these leaves so that these changes remind us here and there of the structure of the gall in pear leaves caused by the mite *Eriophyes piri*, where the strong growth in length of all the mesophyll-cells together with the great enlargement of the intercellular spaces is the well-known symptom of this phytoptosis. But the cells of the spongy parenchyma in the silvering leaves do not remain together a long time; they fall asunder easily, as Güssow and Brooks state. Whole groups of the cells are disintegrated in this way which is the result of the dissolution of the middle lamellae. As is known a marked increase in size in the epidermal cells may also be observed. This is shown in the bulging of the walls of the epidermis towards the palisade cells. The volume of these cells is therefore greater. The tearing off of the epidermis from the palisade cells is very striking. Either the epidermis is simply detached and somewhat elevated so that a closed cavity is formed in this place, or the epidermis is quite torn asunder and the free portions are slightly lifted up from the palisades. Both this formation of cavities and the tearing of the epidermis are very common but not necessarily always present on all the areas attacked. A similar case was described by Miehe (vide 8, p. 478) as follows: "Epidermiszellen, die von *Synchytrium Taraxaci* infiziert worden sind, wuchern gegen das ihnen anliegende Mesophyll vor und drängen es beiseite u. dgl. m."

As for the cavities in the walls of epidermal cells which Percival (12) describes as a symptom of Silver-leaf disease I can state that I have looked for them in vain throughout an extensive series of preparations. In this respect my results agree with those of Güssow and Brooks.

The phenomenon of silvering of foliage which is the only external symptom of this disease is said to be due to the accumulation of air in the above mentioned subepidermal cavities, which interferes with the normal reflection of light. For instance it causes the white colour of the young *Bryum argenteum* leaves and of the white flower-petal, etc. I have had reason to doubt the adequacy of this theory and so I endeavoured to decide its value by the following experiment. The question is not so simple as it seems at first sight. The following experiment should dispel any doubts: the strongly attacked portion of a leaf was cut into small pieces which were then immersed in water in a vessel connected with an air-pump. Into the same vessel were put small healthy pieces of another shape—so as to distinguish them. These tissues were then completely injected with water. If the white colour is solely due to the air in the subepidermal cavities, it should

disappear after injection. The leaves were cut in quite small pieces (about the average size of 2–3 mm. square) so that the water had easy access to the cavities. After the injection all the segments were carefully dried between filter paper and arranged in line, the attacked injected with the attacked non-injected and similarly the healthy injected with the healthy non-injected. Of course all the leaf-segments swelled up in the water. The white colour of the attacked segments was seen to have disappeared somewhat but not entirely so, for the green colour of the injected segments never equalled the bright green of the normal. The former always were a little dim. And moreover a piece of an attacked leaf loses its grey colour somewhat even without injection, viz. when it has merely been immersed for a short time in water. It became less and less till after about 25 minutes. It seems then that the existence of abnormal air spaces will not account completely for the silvered appearance of the leaves. It is very striking also that the spreading of the silvering always began first of all on and around the vascular bundles (veins) and from that it spread over the surface of the leaves. We can perceive in nature itself from the leaves only slightly attacked and also on looking at the photograph of infected twigs given by Güssow (3, p. 386) that the silvering of the leaves very often starts from the region of the veins.

On the above and the following grounds one must then be sceptical as to the "silvering" being really due to the accumulation of air in special subepidermal cavities: (*a*) it is a striking fact that the phenomenon of "silvering" spreads over the blade very often from the veins, above which the epidermis is very seldom separated; (*b*) the subepidermal cavities are not necessarily always present everywhere in the silvered leaves; (*c*) the contents of the epidermal cells and a certain disorganisation in the mesophyll (see below) can hardly fail to have an effect on the coloration of the foliage.

II.

Before dealing with the cytological changes in the mesophyll cells of the diseased leaves, one may consider a typical cell of the healthy mesophyll of a leaf of *Prunus domestica* var. "Victoria." Here we notice the simple normal relations of the cell content which can be observed in every healthy leaf. In the clear transparent cytoplasm lie the chloroplasts, ellipsoid in form, most commonly close to the cell-walls. They usually contain many starch grains which we can easily recognise in preparations stained by the "inverse" method. By other

methods, however, the starch grains appear as structures shining through the thin transparent substance of the chloroplast. The nucleus stains intensely and is more or less spherical or oval and is often found in a somewhat central position in the cell. It usually contains one or two nucleoli and plenty of chromatin substance (Fig. 1).

If we compare with these the mesophyll cells of the "silvered" leaf—either the palisade or spongy parenchyma—we cannot be in doubt as to the difference between the cytological structure of the

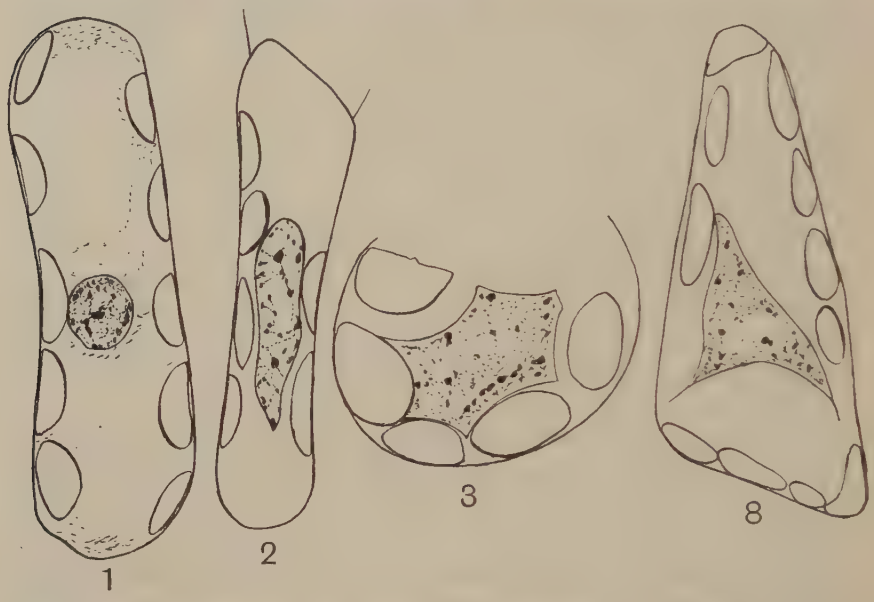


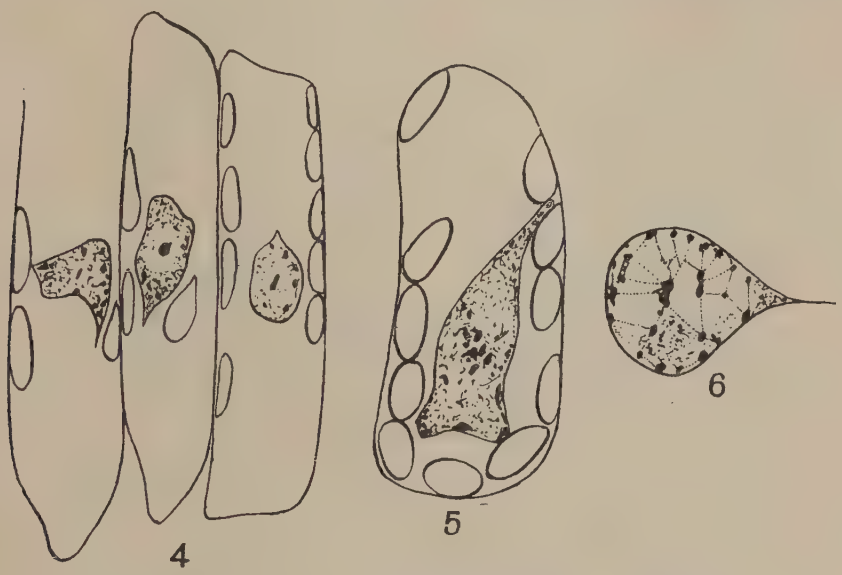
Fig. 1. Normal palisade cell from leaf of *Prunus domestica* var. "Victoria."
Figs. 2, 3, 8. Cells from an affected region of the same leaf, showing a hypertrophied nucleus (fig. 2), a nucleus with depressions corresponding to the surface of the chloroplasts (fig. 3), and a nucleus with filamentous projections (fig. 8).

healthy and the infected tissues. There is often some difficulty in distinguishing the relations in the diseased mesophyll because the intensely stained chloroplasts make observation difficult. But careful study and suitable methods will reveal the true state of affairs.

I am unable to agree with Percival (12, p. 391) in his statement: "The peculiar light grey colour of the leaves is due to these air-filled spaces, and not to any alteration in the chloroplasts: the latter structures are of the same size and appearance as those in healthy leaves." On the contrary I have found changes in the nuclei, cytoplasm and chloroplasts of the affected mesophyll. In the same slide

it is often possible to distinguish the healthy from the diseased part by staining alone. The cells with the disorganised content are wont to stain differently from those with the normal content, for instance the disorganised content of the preparations which are stained by Mann's method is obviously of a darker blue than the neighbouring healthy cells.

The nucleus. The changes in the nuclei which I was able to observe in the mesophyll cells of the diseased leaves are described according



Figs. 4, 5. Palisade cells with nuclei of amoeboid form.
Fig. 6. Nucleus from spongy parenchyma showing filamentous projection.

to their probable developmental stages in the living plant, in so far as it is possible to deduce these from stained preparations. First of all the nuclei become greatly elongated and hypertrophied, their volume is much increased so that they sometimes fill the cell-interior up to the chloroplasts, even touching them (Fig. 2). By this time the surface of the nuclei shows depressions corresponding often to the surface of the nearest chloroplasts (Fig. 3). The nuclei sometimes assume quite an irregular or amoeboid form (Fig. 4) producing lobes which seem to be directed between two neighbouring chloroplasts even when the nuclei do not fill the whole cell-interior (Fig. 5). In other cases the nuclei are seen to produce a long thin projection which is one of the

most remarkable features of disorganisation. Some nuclei send off two such fine projections. These thread-like projections are sent off either from the rounded nucleus or in most cases terminate one or more of the above-described lobes (Figs. 6, 7, 8).

In many other cases the elongated nucleus lies transversely across a palisade cell, giving the appearance of a transverse septum (Figs. 9, 10).

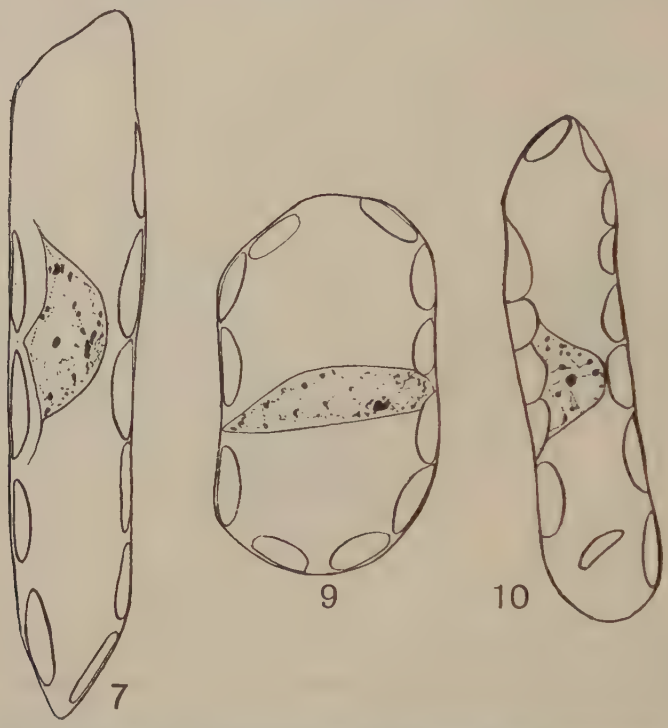


Fig. 7. Palisade cell showing nucleus with filamentous projections.
Figs. 9, 10. Palisade cells showing transverse orientation of nuclei.

And at the same time both ends of the nucleus spread somewhat laterally along the walls so that the nucleus, changed in this way, has two concave surfaces (Fig. 11). Sometimes this septum-like nucleus is of a considerable size so that it fills a third of a cell. The outlines of the septum-like nuclei become in some cases irregular (Fig. 12). While the septum-like nuclei appear in the palisade cells rather frequently, yet this is not the case in the spongy parenchyma. On the contrary, in the latter tissue the nuclei commonly show sharp pointed lobes as was above

described. Probably the form which the disorganised nucleus takes may be dependent on the form of the cells (Figs. 13, 14).

As to the nucleolus, the normal spherical or only slightly deformed nucleus contains one or two easily distinguishable nucleoli. After subsequent disorganisation we cannot discern such obvious nucleoli in the nuclei.

In addition to modifications of form of the nuclei marked changes

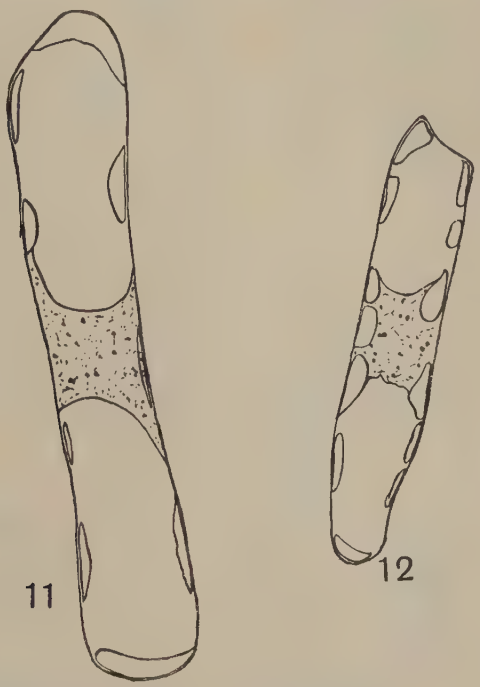
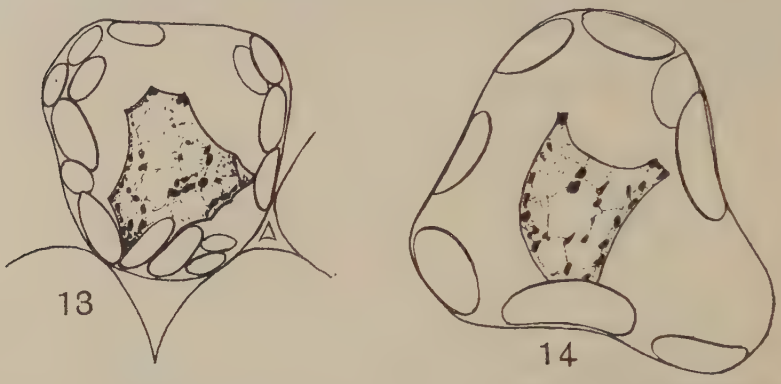


Fig. 11. Palisade cell showing septum-like nucleus.
Fig. 12. Palisade cell showing septum-like nucleus with irregular outlines.

take place in the chromatin. The normal nucleus contains a conspicuous network with numerous intensely stained chromatin granules. But as it disorganises the nucleus always contains fewer and fewer granules. There is strong evidence (see below) for the view that these grains sometimes wander out from the nucleus into the cytoplasm. Cases were found which were a distinct proof of this. The chromatin manifests a tendency to accumulate on the periphery of the nucleus. It is possible that the grains move centrifugally and press against the nuclear membrane, for we notice the chromatin grains accumulated close to

the wall of the deformed nucleus (Figs. 13, 14). Apparently the different forms of the disorganised nuclei are connected with the wandering out of the chromatin grains. Here and there we can observe that the



Figs. 13, 14. Cells from spongy parenchyma showing nuclei with sharp-pointed lobes.

chromatin grains are outside the nucleus (Figs. 15, 16), and what is more a whole line of several chromatin grains lies, in a few cases, outside of the nuclear substance (Fig. 15). In such a case the nuclear

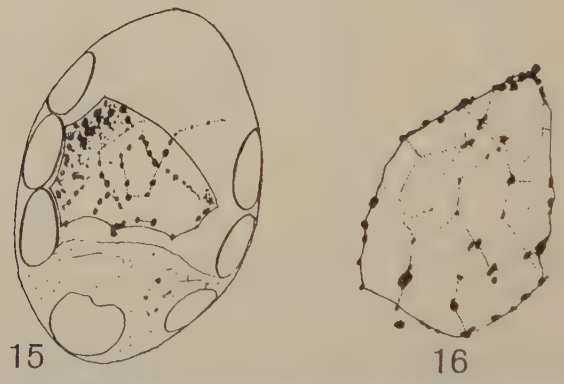


Fig. 15. Cell from spongy parenchyma showing chromatin grains outside the nucleus.
Fig. 16. Nucleus from palisade cell showing chromatin grains accumulated at the periphery.

membrane seems to be very thin; or it disappears entirely in some places, but this is a rare occurrence. Another reason for the view that chromatin grains migrate from the disorganised nucleus is the occurrence in the palisade parenchyma of disorganised nuclei which are without

chromatin grains (Fig. 17). The shrivelled remains—probably dead—of the septum-like nucleus appear as a homogeneous substance (Fig. 17) which stains uniformly and somewhat differently from the nuclear substance in the earlier stages. The migration of the chromatin from

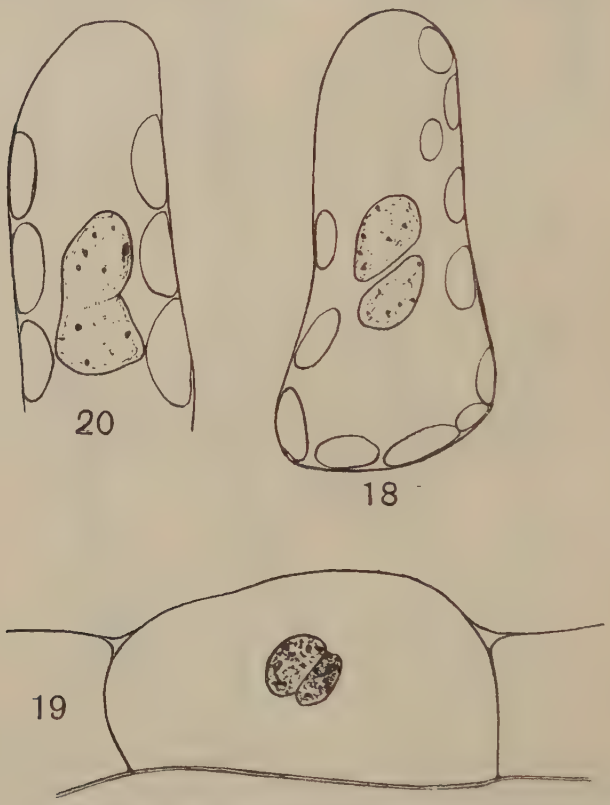
Fig. 17 Cells from palisade tissue. Three of them showing disorganised content; chloroplasts destroyed and mostly vacuolised; remains of nuclei (in the long cells on each side) homogeneous and without chromatin granules. The fourth cell, the short one in the middle below, is normal.

the nucleus was described in *Nematode*-galls by Nemec (11, pp. 171, 483) and by Guttenberg (vide 7, p. 202) in the galls of *Ustilago maydis*.

It is doubtful whether the phenomenon here has any definite relation to mitochondria as they are conceived by Arnoldi[1] (vide 15, p. 707). For the chromatin grains in the cytoplasm just outside of the nucleus very

[1] Arnoldi states that the mitochondrium is the same as the chromidium, viz. that the mitochondrium is of nuclear nature. But this view has very few adherents to-day.

seldom remain as permanent structures in the diseased *Prunus* leaves. They are most probably very quickly dissolved in the cytoplasm, as was found in the *Nematode*-galls by Nemec. Such an outward movement of chromatin grains away from the nucleus is hardly a normal process; it may be a symptom of pathological conditions in the cell, just as is assumed for the *Nematode*-galls by Nemec, who says: "In den



Figs. 18, 19. Binucleate cells from palisade tissue and upper epidermis respectively.
Fig. 20. Part of palisade cell showing constricted nucleus.

Riesenzellen der *Heterodera*-gallen bei *Washingtonia robusta* treten zwar in einem bestimmten Stadium regelmässig fast alle Chromatinkörner aus dem Kern heraus, aber die Kerne degenerieren hierauf, und es scheint daher, dass es sich um einen pathologischen Vorgang handelt" (11, p. 483).

In a few cells I have found in the silvered leaves nuclear "fragmentation" or at least a tendency to it. In several palisade cells and in a single epidermal cell two nuclei occur in close proximity to each

other (Figs. 18, 19). Or the elongated nucleus shows a simple constriction (Fig. 20) which may be the beginning of amitosis. The direct division in the plant tissue is, according to the generally accepted view, a characteristic of degeneration or of pathological conditions. I did not succeed in following the fate of the amitotically divided nuclei. Probably they disorganise in the same manner as the nuclei in the uninucleate cells of the diseased area, since in one binucleate palisade cell at least one nucleus showed obvious traces of early disorganisation. In some other attacked cells—as was described above—it can be definitely established that the nucleus becomes more and more deformed, disorganised and finally dies (Fig. 17).

The chloroplasts. In the diseased area the chloroplasts show remarkable diversity of structure and form, being often very irregular and sometimes destroyed. The beginning of their disorganisation appears as a corrosion of the surface. Their volume is consequently markedly reduced in size and thus in optical section they appear thinner and thinner (Fig. 17). The unusual irregular outlines can be easily observed on the chloroplasts as seen in surface view in a cell uncut by the microtome-knife.

In the strongly affected region the chloroplasts finally appear as very thin scale-like structures, sometimes hardly distinguishable and lying close to the cell walls (Fig. 17). By this time the cell is very often filled with a curious granular substance. In such cells we do not notice any remains of the nuclei. There is no likelihood that the nuclei were removed by the microtome-knife because whole rows of the destroyed cells in such a diseased area do not contain nuclei. Starch grains were never found in the deformed chloroplasts.

The disorganisation does not progress everywhere in the cells in the same way. In some cells the nucleus appears almost normal or only somewhat hypertrophied, while the chloroplasts may be in an advanced stage of disorganisation. On the contrary we notice cells where the partly disorganised nucleus is found in the cell along with healthy chloroplasts. Yet in most cases the disorganisation progresses equally in the two.

There can be no question of the changes described being of the nature of artifacts, for in the immediate vicinity of the disorganised cells we can readily observe others with quite healthy contents (Fig. 17). Also the results of autumn changes are here excluded, because all the described cytological modifications appear in leaves fixed not only in October and September but also in those fixed in July.

The cytoplasm forms a peripheral layer in the healthy mesophyll cells of *Prunus* leaves and is thin and clear, but in affected cells it is clouded. The cell contents of the diseased area are very striking because of a uniformly stained, granular deposit. This fine granulation occurs in the palisade and spongy parenchyma and epidermis alike and sometimes in the intercellular spaces. It is always in the silvered "Victoria" plum leaves a certain indication of a diseased area. It was never found in the cells with entirely normal content. An attempt to determine the nature of this substance by microchemical methods was not very successful. The tests for sugar (Fehling's solution) and for protein (Millon's reagent) gave negative results. Usually the test for tannin (ferric chloride, potassium bichromate) gave negative results also, although occasionally very slight reactions were obtained.

It would appear that the vascular bundles (veins) play a certain part in spreading disorganisation in the mesophyll. It can often be noticed in the sections that the vascular bundles limit the diseased area; cases were observed where on one side of vascular bundle the tissue was more or less profoundly altered, whilst on the other side the cells did not show any symptoms of attack. It is possible that this fact is connected with the observation previously mentioned of the spreading of silvering from the veins.

III.

Discussions and Conclusions.

The facts above described show clearly that the changes occurring in "silvered" leaves of the plum are not confined to the development of air spaces and the separation of cells but are far more profound than was supposed. There is no doubt that in the diseased leaves markedly abnormal physiological conditions exist.

It is true that some of the phenomena described take place only in pathological tissue, while others are known occasionally in healthy tissue also. For let us consider briefly a few features of the silvered leaves which are similar to those in healthy tissue. The abnormal increase in size of the nucleus is—to judge by the manner of its occurrence—connected with the increase of metabolism just as it is for instance in the large nuclei in the glands of animals or in healthy tissue of the seaweed *Antithamnion* (Schiller, 14), where the surface of the nuclei is much increased in size by enlargement and change of form or development of lobes. In the diseased *Prunus* leaves the hypertrophy of the

nuclei means the beginning of a reaction and we cannot decide whether it is response to the increased metabolism only or to the influence of a toxic substance.

Concerning the amitotic mode of division the most recent view is that it is not a phenomenon that appears in healthy tissues unless the latter are old (*e.g.* the well-known case of amitosis in the old cells of *Chara*) or degenerate. Many cases of apparent amitosis have been explained as modified stages of mitosis (Nemec, 10). There are, indeed, authors who state that even in wound tissue the mitotic mode of division regularly takes place (Strasburger, 19, p. 22). On the contrary we often meet with amitosis in the hypertrophied tissue of galls although even in the latter karyokinesis occurs (Küster, 7, p. 200). Amitosis was observed by Guttenberg in *Capsella bursa-pastoris* after infection by *Cystopus* (Albugo) *candidus*, and by Shibata in *Podocarpus*-galls, etc. (Küster, 7, p. 200). Amitotic mode of division—so far as is known—is never followed by cell division, so that bi- or multinucleate cells result. The few cases of amitosis in the silvered *Prunus* leaves are what one would expect in the organs attacked by such a serious disease ("der Milchglanz sei ein absolut sicherer Vorläufer des Todes eines Zweiges," Sorauer, 17, p. 285), where the conditions leading to hypertrophy prevail. Amitosis here is evidently similar to amitosis in galls. The few binucleate palisade cells observed are probably in respect of their origin the result of amitosis.

Some modifications in the cell and nucleus resembling the condition found in *Prunus* leaves have been often brought about by artificial methods. Thus, for example, twenty years ago Klemm (5) investigated the phenomena of disorganisation in the hairs of *Urtica*, *Momordica*, *Tradescantia* and *Trianea* caused by abnormal conditions of temperature, light, electrical action and the influence of acids. In his experiments marked changes in the protoplast appeared particularly in response to electrical action. The nuclei became elongated or in other ways deformed and finally destroyed. The granular appearance of the nuclei changes to a homogeneous ("ein glasiges homogenes Aussehen," p. 688) and the nucleus later collapses completely just as happens in some cases in the silvered leaves (Fig. 17). Otherwise in Klemm's experiments "der Kern erleidet bei der Desorganisation der Zelle allgemein wenig sichtbare Veränderungen" (p. 686). Several years later Nemec (10) obtained interesting modifications of nuclei by the influence of chloralhydrate, some cases of which resemble these in the diseased *Prunus* leaves.

In the production of abnormalities in the protoplast various other factors have been found to play a certain part. The important literature on this subject is contained in the work of Reynolds (13).

In the cases cited various modifications in the protoplast were attained artificially, viz. by the introduction of a definite external agent. When such a factor induces marked degeneration—as *e.g.* in the electrical radiation experiments of Klemm—then we can look upon it as a strong stimulus in the life of the cell. The pathological factors (vapours of chloroform, electrical radiation, etc.) acted *directly from the immediate vicinity of altered cells*. If we notice in the *Prunus* leaves changes of a like magnitude in the cells, then we can judge in an analogous way of a strong stimulus which probably also is acting somewhere in the immediate vicinity. We may judge of it even more inasmuch as the changes observed in the mesophyll cells of *Prunus* remind us strikingly of those which take place in the various galls, so that it is permissible to compare a leaf attacked by Silver-leaf disease with a gall, even according to the definition of Küster (7, p. 2).

Within the last few years cytological investigations have been carried out on the pathological reactions of the diseased plant. The important literature of this subject is to be found in Küster (7, pp. 198–205) and in the recent work of Reynolds (13). The attention of Reynolds was directed toward the reactions of leaf tissue to fungal invasion in the various phanerogamic plants. *Zea Mays* parasitised by *Ustilago Maydis*, *Pirus malus* by *Gymnosporangium* sp. and *Viola cucullata* by *Puccinia Violae* are among the cases examined. There are also included some host plants (*Panicum*, *Smilax*) upon which the cause of the disease is not clear but is certainly of fungal nature. It cannot be doubted that many changes in the *Prunus* leaves attacked by Silver-leaf disease remind us of those described by Reynolds. This author also states that the nuclei in the parasitised leaf tissue are, as a rule, more or less deformed and enlarged, varying from globular to pear- or even crescent-shaped; that the chloroplasts may be affected, at least in shape and size (*Viola* parasitised by *Puccinia*) and the chlorophyll may disappear (*e.g. Panicum*, *Potentilla* by rust, etc); that the cells before collapse are filled with a granular yellowish substance (*Gaylussacia baccata*, *Zea* in epidermis) and that the pathological tissue is sometimes entirely destroyed and killed (*Smilax*, *Castanea*). All these modifications brought about in the cells by influence of a parasite from close proximity *are quite comparable to the changes of the cytological elements in the case of Silver-leaf disease*. The affected plants which formed the subject

of investigations by Reynolds are to be considered as galls (viz. *mycocecidia*) according to the terminology of Küster.

Changes similar to those which occur in *Prunus* leaves are well-known phenomena also in other galls, whether *mycocecidia* or *zoocecidia*. Enormous hypertrophy of the nuclei may occur in all galls and may attain sometimes unusual dimensions. The nuclei of the host plant under the influence of *Synchytrium* (Guttenberg, 4, p. 438) may become 250 times the volume of the normal nuclei. "An enlargement of the nucleus often to double the normal size and often a change of shape to spindle form" was observed also by E. F. Smith (16, II. p. 92) in plant organs attacked by bacterial disease. Also "septum-like" nuclei similar to those which occur in diseased *Prunus* leaves were established by Guttenberg in *Alnus incana* attacked by *Exoascus amentorum* (after Küster 7, p. 202). The pathological nature of the amitosis and also the forcing of chromatin grains toward the periphery of the nucleus, have already been pointed out.

Not only the cytological characteristics but even the anatomical features of the silvered leaves investigated show a cecidiological nature. The study of histogenesis of galls has shown that the abnormal growth of host cells is a symptom of all galls. In the case of the Silver-leaf disease the hypertrophy of the mesophyll is perhaps not very marked, but yet it is quite obvious from a comparison of healthy and affected mesophyll. A similar hypertrophy of the mesophyll was noticed by Kusano in the leaves of *Vicia unijuga* attacked by *Olpidium Viciae* Kus.: "The mesophyll of the diseased spot is hypertrophied with the enlargement and the increasing number of cells" (6, p. 177). The falling asunder of mesophyll cells which has been previously noted in the silvering leaves by all authors appears also in the leaf-gall of *Oligotrophus bursarius* on *Glechoma* (Küster, 7, p. 197). Moreover the epidermis ruptures under the influence of *Synchytrium Taraxaci*, as already stated.

If we review the comparisons just mentioned we must admit that *the affected tissue in the case of Silver-leaf behaves as a parasitised tissue, and on structural grounds belongs to the category of gall-tissue, using gall in the wider sense of the word.* It is true that there is no characteristic common to all galls, but every pathological feature of the Silver-leaf disease finds a parallel in some gall.

Although the study of the etiology of Silver-leaf disease lies beyond the scope of this contribution yet one etiological deduction was of necessity forced upon one during this investigation. According to the

investigations of Percival, Pickering, Güssow, Brooks (cited above) the basidiomycete *Stereum purpureum* is the cause of Silver-leaf disease as was indicated by successful inoculation experiments. In all these experiments the inoculation of the tree by *Stereum* was followed by the external symptom of the silvering of the foliage, but the fungous hyphae were never found in the leaves. I observed also that there is no trace of any hyphae in the silvered *Prunus domestica* leaves. How are we therefore to account for these remarkable phenomena of disorganisation in the plum-leaf? If the mycelium of *Stereum* existed in the leaf, then the changes described would be quite comprehensible. The diseased leaf with its abnormal internal conditions would be classed without any hesitation among the galls, as are the cases described by Reynolds. It is true that a parasite can produce sometimes marked changes in the nucleus even when it is not in immediate contact with the latter; but in the case of Silver-leaf disease the mycelium of *Stereum* when present is far distant. For the *Stereum* spreads—as it was established by the authors mentioned—in the wood elements of root and stem but never in the leaf nor its petiole. Since the attacked leaf behaves exactly as a gall tissue parasitised by an organism living in the tissues, it is difficult to believe a mycelium so remote could be the cause of such changes of nuclei, chloroplasts and cytoplasm.

Not only the changes of the protoplast, but also the spontaneous maceration of the mesophyll tissue is difficult to explain as a result of the action of a distant mycelium. The latter phenomenon, common in the silvered leaves, reminds us somewhat of the results in Richter's (after Küster's *Aufgaben*, etc. 8, p. 462) experiments with narcotics (vapours of camphor), where a similar maceration of tissue was obtained. The falling asunder of cells, as it is known, may be caused in plants parasitised by an organism in these ways; either the parasitic hyphae penetrate and dissolve the cell walls by cytase or the host cells themselves produce the enzyme under the influence of the parasite. If *Stereum purpureum* is the direct cause of the maceration of the leaves, as has been suggested, then a fungous enzyme must be carried up by the vascular bundles from the lower parts of the host. It is unlikely that an enzyme of the nature of a cytase would be so carried since it would cause the destruction of the vascular strands and other tissues *en route*. It is much more probable that some toxin is secreted in the leaves which causes the changes which have been described. The action on the middle lamella of the leaf cells is probably due to an enzyme secreted by the cells as result of poisoning, *i.e.* is an autolytic action.

It is, however, not at all certain that *Stereum purpureum* is really responsible for the Silver-leaf disease. Indeed we find in the literature concerning the subject of this disease a considerable amount of doubt. At the beginning of this paper various views respecting the etiology of this disease were pointed out. Massee (9, p. 66) has failed to find any hyphae in affected plants. Even Brooks has not found Stereum in all cases of this disease. This author examined some silvered plum seedlings which were growing from seeds obtained from healthy trees, and he states: "The silvering was strictly comparable with that which occurs in adult trees. The epidermis was partly free from the underlying palisade tissue and on trying to cut sections of the leaf there was a decided tendency for the mesophyll cells to fall asunder one from the other. There was no evidence of fungal attack in either leaf, stem, or root. As already stated, the seedlings began to recover when given more room in which to grow and upon examining them in August, I saw that the recovery of the foliage to its normal appearance was well advanced. I came to the conclusion that in such a case as this the phenomenon of Silver-leaf was not caused by *Stereum purpureum*" (Brooks, 1, p. 291). Or on the contrary: "...cases have been already mentioned in which Silver-leaf has not resulted although *Stereum purpureum* has made considerable progress in the tissues. I have seen apple and beech trees which have been killed by *Stereum purpureum* in all probability, but with which the phenomenon of Silver-leaf has not been associated" (Brooks, 1, p. 307). In the light of this evidence it is not possible to believe that *Stereum purpureum* is the sole cause of the Silver-leaf disease! Perhaps the inoculation experiments hitherto carried on have been too few or possibly they are not yet complete enough to decide the relation of *Stereum* to this disease. In Bohemia the same external phenomenon, viz. the silvering of foliage, has appeared frequently in recent years on the leaves of sugar beet (*Beta vulgaris*). Of course we must not draw conclusions here from the similarity of the external symptoms, nevertheless it seems to me that the study of this affection, which of course is not caused by *Stereum*, may possibly explain some obscure points connected with the Silver-leaf disease in trees.

Further studies on the cytological changes occurring in diseased plum leaves would be of value. The changes found in leaves in the spring should be studied. Besides the phenomena above described I found bacteria in the diseased plum leaves; thus occasionally in the cells of the parenchyma bordering on the vascular bundles or in the

epidermal cells. I succeeded in staining them by the method of E. F. Smith (16, III. pp. 129, 914). These bacteria may here be of a secondary nature; nevertheless their appearance is a promising subject for further study[1]. I hope to continue along this line later but for the time being I am not able to extend the work.

The question of the Silver-leaf disease is of economic importance especially in England, as the losses caused by it reach a considerable amount. It is not so in Bohemia, where this disease appears frequently, but its virulence is not yet so marked.

The investigation here recorded was carried out at the suggestion and under the direction of Professor V. H. Blackman, and I wish to acknowledge my indebtedness to him for his valuable suggestions and for the interest with which he followed my work. I desire also to thank Mr W. Brown, M.A., of the Imperial College of Science and Technology, London, and Dr Arthur H. Graves of New Haven, Conn. U.S.A., for kindly editing the English text.

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NOTES ON SOME HYMENOPTEROUS PARASITES BRED FROM THE PUPAE OF *CHORTOPHILA BRASSICAE* BOUCHÉ, AND *ACIDIA HERACLEI* L.

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During the course of an investigation of the life-histories of parasites which attack soil insects, and more particularly of the life-history of *Aleochara bilineata*, Gyll., a Staphylinid whose larvae infest the pupae of the cabbage-root fly *Chortophila brassicae*, the following parasites were reared:

A. From the pupae of *Chortophila brassicae*:
   1. *Phygadeuon fumator*, Grav.
   2. *Atractodes tenebricosus*, Grav. (*vestalis*, Hal.).
   3. *Cothonaspis* (*Eucoila*) *rapae*, Westw.

B. From the pupae of the Celery-fly (*Acidia heraclei*).
   4. *Hemiteles crassicornis*, Grav. (= ? *subzonatus*, Grav.).
   5. *Adelura apii*, Curtis.

As the species numbered 1 to 4 do not appear to have been recorded hitherto from these dipterous hosts, the following remarks on them may be of interest.

1. *Phygadeuon fumator*. Morley (*Ichneum. Brit.* vol. II. pp. 98–99) states that this is one of the most abundant of all British insects, and considering its prevalency, it has very rarely been bred. Its previously recorded hosts are: *Mamestra brassicae* (the cabbage moth), *Emphytus serotinus* (a saw-fly), and a dipterous puparium found in carrion. Morley comments on these records as follows: "I suspect it of preying mainly on Anthomyiid diptera": and he informs me (*in lit.*) that he considers the records of the first two hosts to be erroneous. It is therefore interesting to find his suspicion confirmed. From several hundred puparia of the cabbage-root maggot I have obtained only one specimen, a female,

which emerged July 26th, 1914. The host's puparium was jet black and readily distinguishable from the brown unparasitised pupae.

2. *Atractodes tenebricosus*. This species has been recorded from a great number of localities in Great Britain and Ireland; it is probably ubiquitous. Morley (*loc. cit.* p. 247) remarks that he can find no record of its parasitism. From 506 puparia only two specimens were obtained. One female emerged at the end of March, 1914, and one male on May 2nd, 1914.

3. *Cothonaspis rapae* was obtained in abundance from puparia of *C. brassicae*. This Cynipid was first recorded and described by Westwood (*Mag. of Nat. Hist.* vol. VIII. 1835, pp. 171–9) from some examples sent to him for identification; they were obtained from turnips on which dipterous larvae were feeding. Westwood, however, believed that the Cynipid larvae also fed on the turnips and that the Cynipid and the dipteron had no further association with each other. In the light of our present knowledge we now know that Westwood's supposition was based on insufficient data; so few Cynipids, however, were then known to be parasitic that his mistake was quite excusable. Concerning this species Cameron (*Monog. Brit. Phyt. Hymenopt.* vol. III. p. 210) remarks, "bred by Westwood from the tumours on turnips formed by *Ocyptera brassicaria*." There is no mention made of *Ocyptera* in Westwood's article quoted previously, and I have not attempted to trace the source of Cameron's statement[1], but there appears to be some mistake with regard to it, as the Ocypteridae are parasitic diptera attacking, so far as known, Orthoptera, Hemiptera, Coleoptera, and Lepidoptera (Townsend, C. H. T., *Insect Life*, vol. VI. 1894, p. 201). It is therefore very improbable that *Ocyptera brassicaria* could form tumours or galls on turnips. The cabbage-root maggot, however, attacks turnips as well as members of the cabbage family, and I believe the specimens described by Westwood probably emerged from the dipterous puparia whose larvae had been feeding on the turnips. The statement of the observer who sent the insects for identification, that the larvae found feeding on the turnips were "exactly like those in the knobs of cabbages," lends support to the above explanation. Westwood figures a pupa obtained from one of the larvae and, so far as one may judge, it was a pupa of *Chortophila brassicae*. As the Eucoilinae are recorded as attacking Tachinidae, the possibility of *C. rapae* having been bred from *O. brassicaria* is, however, not excluded. There are at least two

[1] Probably specimens so labelled were among Westwood's 1833 types, when these were examined by Cameron in 1888 (cf. *Ent. Mo. Mag.* XXIV. p. 209).—Claude Morley.

generations of *C. rapae* produced each year. One generation emerges in April and May, and the majority of the second generation in August. A few individuals, however, emerge during the later months of the year. I have a record of one specimen which emerged near the end of December, 1914.

4. Of *Hemiteles crassicornis*, one of the two species of parasites obtained from the celery-fly puparia, Morley (*loc. cit.* p. 141) notes that it is doubtless common, and had not then (1907) been bred. I obtained it from puparia collected in October, 1913, in the garden of Mr H. Bury, High Lane, Cheshire, together with—

5. *Adelura apii*, a Braconid, which, according to Marshall (*Monog. of Brit. Bracon.* Pt. VI. pp. 367–8) has been frequently reared from *Acidia heraclei*. Mr Bury sent me thirty-eight celery-fly puparia, and of these thirty-four were parasitised. Three examples of *A. apii* were observed on December 6th, 1913. No further parasites were seen until April 30th, 1914; between this date and May 14th seven *H. crassicornis* emerged, and on August 11th another individual of this species was obtained. Of the eight *Hemiteles* obtained four were females and four males.

In 1914 the first *Adelura* was recorded on May 16th, and subsequently they emerged at intervals of two or three days until June 23rd. Between these two dates 16 specimens were reared. No further examples were noted until August 11th, when another individual was obtained. Twenty *A. apii* were obtained altogether and of these seven were females and nine males; the sex of four individuals was not determined. Two of the celery-fly puparia were opened in January, 1914; they contained fully fed parasitic larvae, and only four celery-flies, which emerged at various times, were obtained altogether. An examination of the remaining puparia was made and four were found to contain dead parasites, of which one was *A. apii*.

From thirty-eight puparia of *Acidia heraclei* were obtained:

4	*Acidia heraclei* (adult flies).
8	*Hemiteles crassicornis*.
20	*Adelura apii*.
2	parasitic larvae.
4	dead parasites which failed to emerge, one of which was *A. apii*.
38	Total.

As may be seen, the number of infected pupae was very large. The

high degree of parasitism is rather remarkable, but the number of pupae from which these records were made is too small to admit of any general conclusions being based on the results obtained. It is, however, worthy of note that last year very few celery plants in Mr Bury's garden were found to be affected by celery-fly larvae, and there may possibly be some relationship between this fact and the high degree of parasitism of the pupae from the last brood of celery-flies of the previous year (1913).

A larger number of celery-fly larvae and pupae were collected in the autumn of 1914 at the Agricultural College, Holmes Chapel, Cheshire, and in the neighbourhood of Northenden, Cheshire, with the object of obtaining results on a larger scale. These larvae and pupae were also found to be heavily parasitised. The complete results of the investigation will not be obtainable, however, until August or September, 1915, owing to the period of emergence of the parasites extending over so many months of the year.

I wish to express my thanks to Mr Claude Morley for naming the Ichneumons (1, 2 and 4), to Professor Dr J. J. Kieffer, of Bitsch, for identifying the Cynipid (3), and to Mr G. T. Lyle, of Brockenhurst, for confirming my identification of the Braconid (5).

NOTE ON AMERICAN GOOSEBERRY MILDEW.

By M. A. BAILEY, B.A.

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During the last four years a large number of seedling gooseberries have been raised here each season in the course of an experimental study of the inheritance of disease-resistance and other characters in this group*.

Many of these seedlings are crosses between English and American types and, as such, are either partly or wholly immune to American Gooseberry Mildew, but the majority were obtained by the self-pollination of various English varieties. The following observations on the incidence of disease refer solely to the latter class.

The first batch of seedlings raised—about a thousand plants—were pricked out in the open (Plot *A*) on May 6th, 1912. They remained free from disease till about the end of June. On August 25th and subsequent days a careful examination of the individual plants was made, and the intensity of infection and relative position of the bushes noted. This examination showed that about 40 % of the plants were infected with mildew, the intensity of the attack varying from "slight" to "very severe." Infected bushes were found in all parts of the plot, and a tendency to occur in groups suggested that the primary infection was sporadic but widespread in its distribution.

The manner in which this primary infection took place is not known but it is possible that it was connected with the presence during the previous year of two infected gooseberry bushes in a rather distant portion of the grounds. These two bushes were destroyed in September, 1911, but by that time some of the perithecia would have already fallen, and ascospores from these may have produced the infection in 1912.

In cases of slight infection the mildew was found chiefly on the underside of the young leaves, and frequently occurred high up on the

* In this work I have had the collaboration first of Mr W. O Backhouse and, subsequently, of Mr J. W. Lesley.

bush. The more heavily infected bushes showed mildew on the young shoots, and an examination of these shoots made at a subsequent date showed perithecia present amongst the mycelium, but the condition of the ascospores in these perithecia was not ascertained.

When the examination of the bushes had been completed, they were sprayed with Liver of Sulphur with the idea of keeping the disease under control, though this wash has since been proved by Professor Salmon to have little or no effect in the case of this particular mildew.

On November 6th all the bushes were transplanted to another plot (Plot *D*) situated about 50 yards from Plot *A* and separated from it by a low hedge. As it was desired to make observations on the natural habit of growth of these seedlings, they were allowed to remain *entirely unpruned*.

The following year (1913) a new batch of seedlings was pricked out during June on a portion of Plot *A*, which had been occupied by the infected seedlings of the previous year.

The summer of 1913 appeared to be unfavourable to the growth of the mildew, and a relatively slight, though more or less general, infection was recorded on the seedlings of Plot *A*. The season was a very dry one, and this probably had some effect, if only through the more rapid ripening of the young wood. The plants in Plot *D*, many of which, as recorded above, had been heavily infected during the previous year, remained entirely free from mildew, with the exception of one or two bushes—less than 0·5 %—which showed very slight traces of disease.

During the winter the plants received rather light pruning.

In May, 1914, the remaining portion of Plot *A* was filled up with newly raised seedlings, those of the previous year being allowed to remain in their places.

Mildew reappeared during the summer in Plot *A*, and in August it was found that almost every one of the one-year-old plants was more or less heavily infected, as also were all those of the current year, which had made sufficient growth. As in the previous year, the plants in Plot *D* remained practically free from mildew, despite the fact that they made a lot of young growth, of the kind which is looked upon as being specially susceptible to attack.

To account for the apparent lack of infection during the summers of 1913 and 1914 of the plants in Plot *D* by oidia from the infected plants in Plot *A*, I can only suggest that the presence of the low hedge between the two plots coupled with the fact that Plot *D* lies more or less to windward of Plot *A* (*D* lies south of *A* and the prevalent wind

is S.W.) has been sufficient to prevent the transportation by wind of all but a relatively few spores.

Mr Rogers, in a note in the January number of this *Journal*, refers to a case in which all the visibly affected wood of diseased bushes was removed, and the bushes transplanted to uninfected ground, with the result that they were free from disease in the following year. In view of the occurrences described above, it seems difficult to resist the conclusion that it was the transplantation and not the pruning which was the important factor in the recovery of these bushes, and it is even possible that they might have remained free if the pruning had been omitted.

Salmon has recorded[1] that if shoots bearing the winter stage of the mildew are removed at the beginning of August and gently tapped over a piece of paper, dozens of perithecia, ripe and in excellent condition, fall from the mycelium.

The above observations tend to confirm his view that the majority of the perithecia fall from the meshes of the mycelium in the late summer and autumn, and that of those which remain throughout the winter very few are viable.

On the other hand, Salmon, in a paper read at the meeting of the Association of Economic Biologists, 1914, refers to the frequency with which the disease reappears first on young berries on the *upper* branches, and to account for this he suggests that in these cases the reinfection has originated from perithecia which had lodged in crevices in the bark or between bud scales, etc. Though, in my experiments, the first raised seedling gooseberries were only about nine months old when moved to their new quarters, the majority had already made considerable growth. In many cases the bark had begun to show longitudinal cracks or even slight flaking, and the plants were, of course, plentifully supplied with bud scales. Also, if the primary reinfection originates from perithecia entangled in this manner, it does not seem clear why the lower branches should not be affected to an equal degree.

It will be seen above that in the case of the first batch of seedlings raised there was a tendency for the mildew to appear more particularly on leaves high up on the bushes, and it seems possible that the explanation of this and of the case described by Mr Salmon may lie in the different atmospheric conditions which obtain at the top and at the bottom of a bush, when its leaves are sufficiently developed. Spores which alight near the top of a bush must be subjected to greater ranges

[1] *Journal of Agr. Sci.*, vol. VI. p. 2, May, 1914.

of temperature and greater degrees of desiccation than those which fall on to leaves growing lower down.

It is possible that these or some similar factors may have a favourable influence on the germination of the spores, thus producing the effect described.

The favourable effect of cooling on the germination of spores of *Cystopus candidus* has been shown very clearly by Melhus[1], and Eriksson and Henning[2], working on the Uredineae, found that aecidiospores which had been placed on ice for a time gave a much higher percentage of germination than those which had been left throughout at the temperature of the room.

[1] Wisconsin Agl. Expt. Station 28*th Ann. Rept.*, 1911.
[2] *Die Getreideroste*, p. 73.

THE EFFECT OF VARIOUS CHEMICALS ON BLOW-FLY.

By W. F. COOPER, B.A. (Cantab.), F.C.S. and W. A. B. WALLING.

(*From the Cooper Laboratory for Economic Research, Watford.*)

Introduction.

The experiments described in the present communication were made with the object of determining the insecticidal effect of various chemicals, a large number of which, hitherto, have never been actually employed as insecticides[1]. Most of these chemicals are already articles of commerce, whilst the remainder, if they should prove to be effective, could be produced on a commercial scale, if the demand arose.

The selection of the chemicals used was purely haphazard, the immediate object being to eliminate the least promising, and to gain such insight as would lead to a later and more precise series of experiments with the more promising compounds; also the range of selection was as wide as possible, and this has been justified by the results, as some of the most efficient compounds were unusual ones.

The choice of a suitable pest on which to work was also a matter for some consideration, especially as it was desired that the preliminary investigation should have as high a practical value as possible.

Some time ago, the authors were investigating the effects of different chemical reagents upon the eggs of Lepidoptera, but, for various reasons, they were forced to the conclusion that *ova* are unsuitable subjects for experiments of this nature, and the work was abandoned. These objections do not apply to the *larva*, and the question resolved itself

[1] Since this paper was written, the almost universal state of war has created conditions which are unprecedented in their possibilities for the propagation of disease by flies. In those areas which are at present the scene of military operations, the problem of dealing with flies, as agents in the transmission of disease, is likely to become acute in the immediate future; and we venture to hope that our results, incomplete as they are, may possibly afford some useful suggestions to those who are undertaking an active campaign against this menace.

into the choice of a form which was convenient to handle, and obtainable in quantity through the greater part of the year. Muscid larvae conform to these conditions, and as insecticidal methods of dealing with these, particularly in connection with the maggot-fly pest of sheep, and the dissemination of disease by house-flies, have been the subjects of much attention in recent years, one or other of these appeared to be eminently suitable.

The problem of the Maggot-fly pest is one which specially appeals to us, and, as the subject of the relation of the House-fly to the transmission of disease has been adequately dealt with by others, it was decided to use the larval form of one of our English Maggot-flies as a subject for experiment. The larva of *Calliphora vomitoria*, the common Blue-bottle, is readily obtainable at all times from dealers in anglers' requisites and consequently was selected as the most convenient species.

We realise that the most satisfactory manner of attacking the Maggot-fly problem would be in the nature of field experiments on the actual species, but the preliminary sorting out of likely chemicals is carried out most conveniently in the laboratory, so that it is only necessary to experiment with a small selection when the opportunity for field experiments presents itself.

The most obvious means of protecting sheep against the ravages of the Blow-fly are (*a*) the application to the fleece of some substance repellent or distasteful to the flies; Lavender Oil has been recommended in this category: (*b*) the application to the fleece of some compound actually poisonous to the Blow-fly or its larva. Our experiments fall, therefore, under these two main divisions.

As is usual in this class of investigation, very variable and contradictory results have been encountered. These are due to unknown or uncontrollable conditions, and such sources of error can only be eliminated by repetition of the experiment under slightly varying conditions.

The appearance of a Chalcid fly, which attacked the larvae in our later experiments, and, it is to be feared, seriously vitiated the results, unfortunately prevented us from confirming many of the results obtained, and this must be our excuse for publishing results obviously incomplete, and, in many respects, unsatisfactory. It is hoped, nevertheless, that the results obtained will serve as some guide to further work, especially in the direction of field experiments.

The actual manner in which the Blow-fly larva is killed by an insecticide, whether by absorption through the skin, ingestion, by suffocation due to the blocking of the spiracles, or by some other means,

though a very important question, is outside of the province of this paper.

The Maggot-fly species, chiefly responsible for the damage to sheep in Australia, are *Calliphora oceaniae*, *C. villosa*, *C. rufifacies*, and *Lucilia caesar*. It is impracticable to employ any of these species for experimental work in this country, and, as the exact species is of little importance in preliminary work, for reasons specified above, the larva of the common "blue-bottle" (*Calliphora vomitoria*) was used.

At first, the larvae were all bred from flies captured locally. Large pieces of horse-flesh were exposed in a room, suitable precautions having been taken to prevent the escape of the flies, and a moderate supply of larvae was readily obtained. Owing, however, to the large number of chemicals for trial, this supply of maggots was inadequate, and larger supplies had to be obtained from a dealer in angling requisites. Buckets of cow manure had been placed in the breeding room, as it was thought that this might hasten the development of the flies, and, as prior to the introduction of purchased maggots and the cow manure, healthy larvae were always obtained, we suspect one or other of these as the source of the *chalcid* infection which upset our experiments. The mortality in our controls so long as we were using home-reared larvae was usually very low, and rarely exceeded 10 %. In the later experiments, however, the mortality in the controls increased to an excessive degree, and in not a few cases exceeded that of the chemically treated larvae! The *chalcid* infestation increased to such a degree, that very few of the pupae developed, and the investigation had to be abandoned[1].

A. *Experiments with Compounds presumably deterrent to the Adult.*

For this purpose, slabs of horse-flesh, of about 1 lb. in weight, were placed in shallow cardboard boxes, and the exposed surface dusted over with the reagent, suitably diluted with precipitated chalk. The boxes were then exposed to the flies. They were examined daily, and careful note taken of any "blowing." Controls, of untreated meat, were similarly exposed.

[1] The species of *Chalcid* fly which caused the trouble is unknown. Specimens were sent to the British Museum for the purpose of determination, but were reported as unknown.

The astounding mortality caused by this infection suggests a means of controlling the Blow-fly pest in pastoral countries, and we have since heard that Mr Froggatt, Chief Entomologist to the New South Wales Government, is carrying out experiments with this end in view.

The following table gives the results of this experiment.

TABLE I.

The chemical was diluted with precipitated chalk, dusted on to the slabs of horse-flesh, which were then exposed to the Blow-flies for a period of 17 days. + signifies blown.

Substance	% of reagent in powder	Result	No. of days before flesh was blown
Zinc Oxide	10·0	+	7
Copper Carbonate	10·0	+	13
Sulphur	10·0	+	17
Arsenic Sulphide	10·0	+	11
Nitrobenzene	3·3	+	11
Eucalyptus	2·2	+	11
Clove Oil	1·7	+	11
Turpentine	1·6	+	7
Amyl Acetate	2·0	+	7
Methyl Salicylate	1·5	—	—
Cedarwood Oil	1·6	+	7
Oil of Camphor	2·0	+	13
Pyridene	2·1	+	7
p-Nitraniline	3·3	—	—
Aniline	1·7	+	7
p-Nitrophenol	3·3	+	7
Trichlorphenol	3·3	+	7
o-Nitrophenol	3·3	+	7
β-Naphthylamine	3·3	+	7
β-Naphthol	3·3	+	17
Oxalic Acid	10·0	+	11
Borax	10·0	+	17
Picric Acid (moist)	1·0	—	—
Creosote	4·4	—	—
Green Oil	4·1	—	—
Boracic Acid	10·0	—	—
Fusel Oil	1·15	—	—
Pine Oil	1·15	—	—
Alizarine Oil	1·2	—	—
Origanum Oil (brown)	1·0	—	—
Mustard Oil	1·0	—	—
Sod Oil	1·3	—	—
Lavender Oil	0·16	+	6
Aniseed Oil	0·33	+	6
Ginger	1·0	+	6
Iodoform	0·7	—	—
Dimethylaniline	2·2	—	—
Quinoline	1·5	—	—
Butyric Acid	1·8	+	6
Saxin	0·07	+	6
Allyl Alcohol	1·0	—	—
Alion	1·6	—	—
Saponin	10·0	—	—

Controls all badly blown within seven days.

As a result of this series of experiments, it is evident that a very considerable number of chemicals are deterrent to the flies and are capable of protecting horse-flesh from their ravages. Later in the summer, when the flies were much more numerous, this series of experiments was repeated with the same powders. In this case, all controls became blown in one day, and the protective effect of the powders was not nearly so marked as in the first series. Most of the pieces of flesh became blown in one to four days, immunity being only conferred by the powders containing the following chemicals: copper carbonate, nitrobenzene, borax, picric acid (calcium carbonate), creosote, sinapis oil, and aniseed oil.

The exact means by which this immunity is conferred is unknown. Clearly the sense organs of the fly itself are affected. Assuming the fly selects a spot for egg-laying from the smell, *e.g.* of decaying meat, *i.e.* to be guided by chemotaxis affecting "smell organs," these compounds influenced the smell of the horse-flesh by the flies. The deterrent compounds may have acted solely as preservatives and so prevented the decay of the horse-flesh that the flies failed to recognise it. On the other hand, either the taste or the smell of the compounds themselves may have been obnoxious to the flies.

It may be pointed out that the experiments in this section deal with chemicals obnoxious to the *adult fly*. The following sections have to do with chemicals obnoxious or toxic to the *larvae*. The results therefore are not comparable.

B. *Experiments with Compounds, presumably toxic to the larvae.*

The very large scale on which a successful insecticide would have to be applied precludes the use of a pure chemical and necessitates its dilution either by solution, emulsion in a suitable vehicle or admixture with an inert powder. Obviously the vehicle must be cheap and non-injurious to the sheep or its fleece. The following suggest themselves as being suitable in this respect: water, or paraffin as liquid vehicles, and some such material as precipitated chalk for powders. Many chemicals soluble in an oil but insoluble in water might be applied in the form of an emulsion. On account of its property of "creeping," paraffin might prove valuable. It is a solvent of many chemicals and its price would not be prohibitive, especially if it were applied in a fine spray or as an emulsion.

Precipitated chalk also appears to be quite practicable and has

important properties for the purpose. It is extremely fine, and experiments carried out in the laboratory suggest that it could be blown into the wool as a spray. Oils are readily absorbed by precipitated chalk, consequently this is a very useful method of diluting them. Non-acid solids in powder form can be mixed with it. Chalk is quite neutral and in no way affects the wool. It would not, like lime, combine with soap, so that the treated wools would scour well and dye evenly. Precipitated chalk is obtained in large quantities as a waste product in the process of water softening and costs little more than freightage.

In Australia, water is not altogether desirable as a vehicle for treating sheep; it is not always very plentiful and it sometimes causes damage to the wool, which is usually long just at the time when it is necessary to apply the remedy. There is also the difficulty in wetting the fleece with an aqueous solution, but this is overcome to a considerable extent by the addition of an emulsion.

The use of an active chemical dissolved in an inert oil which is then emulsified by the addition of soap or some other emulsifying agent has certain advantages. It affords a means of applying chemicals which are insoluble in water. The emulsified liquid would also possess a high wetting power, a most desirable property where a greasy fleece is concerned.

Many substances are efficient insecticides in the form of vapour, and as the larvae breathe through spiracles, the method appeared to be worthy of trial. The treatment of lung-worm infection in sheep by inhalation of suitable vapours has been practised with some success in S. America and it would appear that some modification of this process might also be applied to the treatment of the fly pest, though the numbers of sheep to be treated might render it impracticable. A few experiments were carried out with the object of observing the effect of various vapours on larvae.

In determining the susceptibility of the larvae to various chemicals, therefore, our experiments fall under three heads:

(*a*) Those with a powder basis.
(*b*) Those with emulsions.
(*c*) Those with vapours.

It is obviously impossible in this country to carry out experiments on living sheep with the very large number of chemicals involved. It was, however, desirable that our laboratory experiments should simulate natural conditions so far as possible, and, for this reason, our earlier experiments were carried out on pieces of fresh sheep-skin. The skin

was cut into rectangular pieces 12 × 8 inches and these were nailed on to a large board, wool uppermost, strips of wood being nailed between the different pieces, to prevent the larvae from migrating from one to another. The chemical to be tested was mixed with a definite proportion of precipitated chalk, a piece of hide dusted with the medicated powder and a definite number of larvae placed upon it. The hide was then covered with a piece of muslin, which was tacked down all round to the wooden partition strip, and the whole then placed aside for the larvae to develop. Untreated pieces of skin were also set aside to act as controls. The results are given in Table II.

TABLE II.

A definite number of larvae were placed on a piece of sheep-hide, the wool side of which had been previously treated with the active substance diluted with precipitated chalk.

Substance	% reagent in powder	% of larvae not pupating
1. Zinc Oxide	10·0	68
2. Copper Carbonate	10·0	14
3. Sulphur	10·0	0
4. Arsenic Sulphide	10·0	66
5. Nitrobenzene	·03	38
6. Eucalyptus	·02	20
Control for expts. 1–3	—	12
Control for expts. 4–6	—	6

The method was not successful; the pieces of skin dried up and often putrefied. Further, the larvae ate their way through the skin and crawling beneath it, out of the reach of the powders, pupated there in a manner quite impossible under natural conditions. Considerable difficulty was experienced in obtaining supplies of fresh skin suitable for the purpose. The length of the fleece varied very greatly on different skins, this, of course, introducing another undesirable factor. It was evident that, for comparative results, this method was useless and that some substitute for sheep-skin must be found.

Though differing greatly from wool, sand and sawdust appeared to be most convenient for the purpose. Definite quantities of these can be employed, and they are readily mixed with the substance under investigation. It was thought advantageous to make experiments both with sand and sawdust, as they differ considerably in one very important point, which might—and evidently did—have some effect on the results;

namely, the power of taking up or "adsorbing" substances. This property is possessed by sawdust but not by ordinary coarse sand. Wool has this property of adsorbing substances to a marked degree: further, this property is selective, inasmuch as wool adsorbs basic compounds more readily than acidic ones, the dyeing of wool being based upon this property. For this reason many basic substances are included in our list of possible toxic agents as pyridine, aniline, nicotine, β-naphthylamine, *p*-nitraniline.

(*a*) *Powder experiments.*

A definite number of larvae were shaken with the powder so as to be well covered with it, then carefully transferred to a glass jar containing sand or sawdust. The jar was covered with muslin and the number of flies which developed noted. The results are given in Table III. The powder consisted of the toxic agent diluted with dry precipitated chalk; the percentage of the toxic agent in the powder is given in column 2.

TABLE III.

Showing the different effect of sawdust and sand basis. Larvae shaken in powder and then placed in sawdust or sand.

Powder	% reagent in powder	Sawdust basis Mortality % Expt.	Sawdust basis Mortality % Control	Sand basis Mortality % Expt.	Sand basis Mortality % Control
Zinc Oxide	10·0	28	8	32	36
Copper Carbonate	10·0	24	8	36	36
Sulphur	10·0	4	8	24	36
Arsenic Sulphide	**10·0**	**88**	**8**	**68**	**36**
Nitrobenzene	3·3	16	8	28	36
Eucalyptus	2·2	28	8	20	36
Oil of Cloves	1·7	4	8	52	36
Turpentine	1·6	8	8	12	12
Amyl Acetate	2·0	4	8	32	12
Methyl Salicylate	1·5	4	8	8	12
Cedarwood Oil	1·6	8	4	12	12
Oil of Camphor	2·0	20	4	12	12
Pyridine	2·1	8	4	24	8
p-Nitraniline	3·3	16	4	20	8
Aniline	1·7	4	4	4	8
p-Nitrophenol	3·3	12	4	8	8
Trichlorphenol	3·3	8	8	12	8
o-Nitrophenol	3·3	20	8	16	8
β-Naphthylamine	3·3	8	8	0	16
β-Naphthol	3·3	0	8	4	16

TABLE III (*continued*).

Powder	% reagent in powder	Sawdust basis Mortality % Expt.	Sawdust basis Mortality % Control	Sand basis Mortality % Expt.	Sand basis Mortality % Control
Oxalic Acid	10·0	12	8	0	16
Borax	10·0	12	8	8	16
Picric Acid (moist)	1·0	8	4	4	16
Creosote	4·4	0	4	32	16
Green Oil	4·1	4	4	12	0
Boracic Acid	10·0	8	4	52	0
Fusel Oil	1·15	4	4	36	0
Pine Oil	1·15	24	4	0	0
Alizarine Oil	1·2	4	4	12	0
Origanum Oil (brown)	1·0	4	4	0	0
Sinapis Oil	1·0	8	4	12	8
Sod Oil	1·3	8	4	24	8
Oil of Lavender	0·16	12	4	12	8
Aniseed Oil	0·33	8	4	20	8
Ginger	1·0	16	8	24	8
Iodoform	0·7	12	8	12	8
Dimethylaniline	2·2	16	8	4	8
Quinoline	1·5	8	8	8	8
Butyric Acid	1·8	0	12	20	8
Saxin (in alc.)	0·07	4	12	8	8
Allyl Alcohol	1·0	64	12	0	8
Aloin	1·6	4	12	8	8
Saponin	10·0	4	12	—	—

The results obtained in this series of experiments are extremely erratic; this might conceivably be chiefly due to two causes.

(*a*) Death might be due to starvation; the high mortality in the controls pointed to this;

(*b*) Larvae of mixed ages were employed, and these might have very different powers of resistance towards the toxic agent.

The next series of experiments was devised to test these two points. The young and old larvae were kept apart and experiments carried out on each. As before, a definite number were shaken with the powder and then placed in a glass jar in sand, meat being added in each case to prevent death by starvation.

The results are shown in Table IV.

TABLE IV.

Showing the different powers of resistance of old and young larvae. Larvae rolled in powder and then placed on sand.

Powder	% reagent in powder	Young larvae Mortality % Expt.	Young larvae Mortality % Control	Old larvae Mortality % Expt.	Old larvae Mortality % Control
Zinc Oxide	10·0	56	0	0	4
Copper Carbonate	10·0	16	0	0	4
Copper Carbonate	10·0	16	0	0	4
Sulphur	10·0	88	0	4	4
Arsenic Sulphide	**10·0**	**100**	**0**	16	4
Arsenic Sulphide	1·0	64	0	8	4
Nitrobenzene	**3·3**	**100**	**0**	16	4
Nitrobenzene	1·0	44	0	0	4
Eucalyptus	**2·2**	**100**	**0**	8	4
Eucalyptus	10·0	16	0	0	4
Oil of Cloves	1·7	20	0	12	4
Oil of Cloves	5·0	20	0	12	4
Turpentine	10·0	20	0	12	4
Methyl Salicylate	**10·0**	**100**	**32**	—	—
Cedarwood Oil	**1·6**	**100**	**32**	0	20
Oil of Camphor	2·0	44	32	—	—
Pyridene	5·0	20	32	4	20
Pyridene	10·0	12	32	12	20
***p*-Nitraniline**	**3·3**	**100**	**32**	4	20
o-Nitrophenol	3·3	84	28	8	4
β-Naphthylamine	**3·3**	**100**	**28**	40	4
β-Naphthol	10·0	20	28	4	4
Oxalic Acid	**10·0**	**100**	**28**	56	4
Borax	**10·0**	**100**	**28**	20	4
Picric Acid (moist)	**10·0**	36	28	**100**	**4**
Oil of Lavender	0·16	60	12	0	0
Oil of Lavender	1·0	84	12	68	40
Aniseed Oil	0·33	24	12	0	0
Aniseed Oil	1·0	0	12	0	0
Ginger	1·0	96	12	4	0
Ginger	5·0	0	12	0	4
Dimethylaniline	**2·2**	40	16	**100**	**4**
Quinoline	**1·5**	**100**	**16**	20	4
Allyl Alcohol	1·0	96	16	0	4
Allyl Alcohol	0·5	20	16	0	4
Aloin	1·6	20	16	0	4

The results are still somewhat erratic, but it is evident that the young larvae are far more susceptible to the influence of the poisons

than are the old larvae. This shows that it is undesirable to carry out experiments with larvae of mixed ages, and suggests that in preventive measures against the blow-fly pest it is necessary that the remedy should be applied before the larvae hatch, or in the very earliest days of the larval stage.

The higher mortality of the young larvae may possibly be explained as follows. Young blow-fly larvae secrete a fluid which digests tissue. The resulting liquid is then re-absorbed. The toxic effect of a toxic compound, *e.g.* arsenic sulphide, on young larvae may therefore be conceivably due to an interference with their digestive faculties, and, in this case, the compound would be acting as a stomach poison, without, however, entering the larva. The results with young larva would thus be comparable to those obtained with caterpillars, when *e.g.* their digestive organs had been affected. Old larvae, ceasing to feed, no longer secrete digestive fluid, so that the effect of the compound on these would be confined to that of a contact poison, and would chiefly act upon the respiratory organs.

Table V gives the results of further experiments which only differ from those recorded in Table IV by the fact that the powder under investigation was mixed with sufficient sand to reduce the proportion of toxic agent to one-tenth of the original strength. A definite number of larvae were placed in the mixture of sand and powder. As before, the mixture was kept in glass jars covered with muslin. The series contains experiments on young, and old, larvae.

The experiments on the old larvae were for the most part vitiated by the Chalcid infection, to which reference has already been made. A great mortality, sometimes amounting to 100 %, was observed in the control experiments and can only be explained by this fact. Only those experiments in which the controls are not apparently affected appreciably by the parasitic fly are therefore recorded.

The higher susceptibility of the young larvae is most marked and, considering the small percentage of toxic substance present, many of the substances used gave results which lead us to believe that they are worthy of a practical trial in the field. Arsenic sulphide, nitrobenzene, methyl salicylate, cedarwood oil, *p*-nitraniline, borax, picric acid, dimethylaniline, quinoline, as in the previous series, have all given highly satisfactory results; whilst, in addition, copper carbonate, oil of cloves, turpentine, β-naphthol, creosote, green oil, boracic acid, fusel oil, sinapis and aniseed oil, all seem to have a poisonous effect on the young larvae.

TABLE V.

Powder mixed with sand and larvae placed in it.

Powder	% reagent in sand basis	Young larvae Mortality % Expt.	Young larvae Mortality % Control	Old larvae Mortality % Expt.	Old larvae Mortality % Control
Zinc Oxide	1·0	68	0	—	—
Copper Carbonate	1·0	84	0	—	—
Copper Carbonate	**1·0**	**100**	**0**	0	0
Sulphur	1·0	36	0	—	—
Arsenic Sulphide	**1·0**	**100**	**0**	—	—
Arsenic Sulphide	**0·1**	**100**	**0**	68	0
Nitrobenzene	**0·33**	**100**	**0**	—	—
Nitrobenzene	**0·1**	**100**	**0**	**80**	**0**
Eucalyptus	0·22	40	0	—	—
Eucalyptus	1·0	0	0	0	0
Oil of Cloves	0·17	76	0	—	—
Oil of Cloves	**0·5**	**100**	**0**	**76**	**0**
Turpentine	0·16	0	0	—	—
Turpentine	**1·0**	**100**	**0**	16	0
Amyl Acetate	0·17	52	0	—	—
Methyl Salicylate	**0·15**	**88**	**0**	—	—
Methyl Salicylate	1·0	—	—	16	0
Cedarwood Oil	**0·16**	**92**	**0**	—	—
Oil of Camphor	0·17	20	0	—	—
Pyridene	0·17	80	0	—	—
Pyridene	0·5	12	0	0	0
Pyridene	1·0	32	0	0	0
p-Nitraniline	0·33	40	0	—	—
***p*-Nitraniline**	**0·5**	**100**	**0**	0	0
Aniline	0·17	40	0	—	—
p-Nitrophenol	0·33	60	0	—	—
Trichlorphenol	0·33	52	0	—	—
o-Nitrophenol	0·33	96	0	—	—
β-Naphthylamine	0·33	48	0	—	—
β-Naphthol	0·33	48	0	—	—
***β*-Naphthol**	**1·0**	**100**	**0**	20	0
Oxalic Acid	1·0	52	0	—	—
Borax	1·0	96	0	—	—
Picric Acid (moist)	0·1	88	0	—	—
Picric Acid (moist)	**1·0**	**100**	**8**	0	0
Creosote	**0·44**	**100**	**0**	—	—
Creosote	0·1	28	8	76	0
Creosote	**1·0**	**100**	**8**	**100**	**0**
Green Oil	**0·4**	**88**	**0**	—	—
Boracic Acid	**1·0**	**100**	**0**	—	—
Fusel Oil	**0·115**	**100**	**0**	—	—
Pine Oil	0·115	20	0	—	—
Pine Oil	**0·5**	**92**	**8**	32	0
Alizarine Oil	0·12	0	0	—	—
Origanum Oil	0·11	16	0	—	—
Sinapis Oil	**0·11**	**84**	**0**	—	—
Sinapis Oil	**0·5**	**100**	**0**	36	0
Sod Oil	0·13	64	0	—	—
Sod Oil	0·5	88	0	0	0
Oil of Lavender	0·016	60	0	—	—
Oil of Lavender	0·1	96	0	0	0
Aniseed Oil	0·033	36	0	—	—
Aniseed Oil	**0·1**	**100**	**0**	12	0
Ginger	0·1	—	—	0	0
Ginger	0·5	24	0	0	0
Iodoform	**0·07**	**100**	**40**	—	—
Dimethylaniline	**0·22**	**100**	**40**	—	—
Quinoline	**0·15**	**100**	**40**	—	—
Butyric Acid	0·18	52	40	—	—
Saxin (in alc.)	0·007	36	40	—	—
Allyl Alcohol	0·1	24	40	—	—
Allyl Alcohol	**0·05**	**80**	**8**	0	0
Aloin	0·16	76	40	—	—

TABLE VI.

Powder mixed with sawdust and larvae placed in it.

Powder	% reagent in sawdust basis	Young larvae Mortality % Expt.	Young larvae Mortality % Control	Old larvae Mortality % Expt.	Old larvae Mortality % Control
Zinc Oxide	1·0	32	46	0	36
Copper Carbonate	1·0	56	46	44	36
Copper Carbonate	1·0	40	46	4	36
Sulphur	1·0	80	46	60	36
Arsenic Sulphide	1·0	92	46	92	36
Arsenic Sulphide	0·1	8	46	12	36
Nitrobenzene	**0·33**	**100**	**46**	**92**	**36**
Nitrobenzene	0·1	20	46	12	36
Eucalyptus	0·22	72	46	44	36
Eucalyptus	1·0	0	46	8	36
Oil of Cloves	**0·17**	**80**	**46**	0	36
Oil of Cloves	0·5	8	46	16	36
Turpentine	0·16	36	46	16	36
Turpentine	1·0	16	46	12	36
Amyl Acetate	0·17	44	46	76	36
Methyl Salicylate	0·15	40	46	56	36
Cedarwood Oil	0·16	24	46	44	36
Oil of Camphor	0·17	64	46	52	36
Pyridene	0·17	64	46	64	36
Pyridene	0·5	28	46	4	36
Pyridene	1·0	0	46	16	36
p-Nitraniline	0·33	16	46	88	36
p-Nitraniline	0·5	16	46	0	36
Aniline	0·17	16	46	60	36
p-Nitrophenol	0·33	68	46	48	36
Trichlorphenol	0·33	52	46	48	36
o-Nitrophenol	0·33	28	46	84	36
β-Naphthylamine	0·33	40	46	96	36
Naphthol	0·33	0	46	0	36
Naphthol	1·0	0	46	16	36
Oxalic Acid	1·0	28	46	76	36
Borax	1·0	28	46	20	36
Picric Acid (moist)	0·1	36	46	20	36
Picric Acid (moist)	1·0	12	46	12	36
Creosote	0·44	80	46	100	36
Creosote	0·1	16	46	60	36
Creosote	1·0	36	46	8	36
Green Oil	0·4	44	46	40	36
Boracic Acid	1·0	40	46	44	36
Fusel Oil	0·115	28	46	40	36
Pine Oil	0·115	40	46	24	36
Pine Oil	0·5	20	46	0	36
Alizarine Oil	**0·12**	**92**	**46**	20	36
Origanum Oil (brown)	0·11	70	46	60	36
Sinapis Oil	0·11	16	46	0	36
Sinapis Oil	0·5	56	46	0	36
Sod Oil	0·13	36	46	0	36
Sod Oil	0·5	16	46	28	36
Oil of Lavender	0·16	20	46	40	36
Oil of Lavender	0·1	12	46	4	36
Aniseed Oil	0·033	20	46	16	36
Aniseed Oil	0·1	8	46	4	36
Ginger	0·1	24	46	52	36
Ginger	0·5	24	46	0	36
Dimethylaniline	0·22	76	46	**100**	**36**
Quinoline	0·15	52	46	52	36
Butyric Acid	0·18	16	46	56	36
Saxin (in alc.)	0·007	28	46	40	36
Allyl Alcohol	0·1	40	46	8	36
Allyl Alcohol	0·05	32	46	4	36
Aloin	0·16	36	46	20	36

Table VI is a summary of the results of a further series of experiments which were a duplicate of those recorded in Table V, except that sawdust was substituted for sand.

The figures given for the controls in the experiments on the old and young larvae represent the average mortality in *seven* control experiments, each on 25 larvae. There was, however, a very marked variation in the mortality of the controls themselves, and, as this had not been observed to any great degree before the advent of the Chalcid fly, is to be attributed to the ravages of the latter. The very frequent discrepancies, obtained in this series, in which the mortality in the experiment is markedly lower than in the control, may also in many cases be caused by the Chalcid fly. Comparing the results on *sawdust* with those on *sand*, it is at once evident that the toxic agents are not nearly so effective in the case of the former. This is merely a confirmation of a result obtained in an earlier series of experiments, but it is of importance as indicating that the high toxic values, which various substances show with sand, would in all probability be reduced in actual practice, owing to the relatively higher adsorptive powers of the fleece.

The highly poisonous nature of arsenic sulphide, nitrobenzene and creosote is again confirmed in this series of experiment.

(*b*) *Experiments with emulsions.*

The experiments with emulsions include most of the compounds which previous series of experiments have shown to be fairly efficient, together with some new preparations.

The actual experiments were carried out as follows:

40 gms. of sawdust were taken and sprayed with an emulsion containing 1 % of the active constituent. Sufficient liquid was used to make the sawdust just damp, the sawdust being well mixed during the spraying. Fifty larvae were then placed in the sawdust, in a glass jar, the mouth of which was covered with a piece of muslin. The results are given in Table VII.

Satisfactory results have only been given by two of the preparations, namely those containing safrol, and β-naphthol and sulphur. The general results are not nearly so good as those given by the powders and the series was therefore not extended further, except that some of the same poisons were tried at a higher concentration. The whole of these experiments were, however, so badly infested with the Chalcid fly that the results were useless.

TABLE VII.

Emulsions.

Reagent	Mortality % Expt.	Control
Creosote	16	12
Turpentine	8	12
Methyl Salicylate	24	12
Crude Pyridine	30	12
Safrol	**92**	**12**
Indol	16	0
Preparation containing Green Oil and Pyridine	50	8
Preparation containing Sulphides of carbon and potash ..	0	8
Preparation containing β-Naphthol	2	8
Preparation containing Green Oil	20	8
Preparation containing Resin Oil	18	8
Preparation containing β-Naphthol and Potassium Sulphide	.4	8
Preparation containing β-**Naphthol and Sulphur**	**100**	**8**
Preparation containing Potassium Sulphide	14	8

(c) *Vapour experiments.*

In these experiments a large number of larvae were placed in a wide glass tube, about $1\frac{1}{2}$ ins. in diameter and 9 ins. long, one end of which was attached to an aspirator and the other to a "U" tube containing the material to be tested; so that the air which was drawn over the larvae by the aspirator became saturated with vapour in the "U" tube. The larvae were subjected to this treatment for $3\frac{1}{2}$ hours; then they were placed in sawdust in jars.

The controls were placed in tubes through which pure air was drawn in the same manner.

TABLE VIII.

Vapour Experiments.

Reagent	Mortality % Expt.	Control	Duration of exposure to vapour h.	m.	Remarks
Benzene	22	10	4	30	
Xylol	36	10	4	30	
Aniline	6	10	5	—	
Acetone	62	10	5	—	
Nicotine	10	16	3	30	
Nitrobenzene	42	16	3	30	
Clove Oil	12	16	3	30	
Eucalyptus	8	16	3	30	
Turpentine	16	14	3	30	
Amyl Acetate	12	14	3	30	
Sinapis Oil	8	14	3	30	Oil frothed freely.
Cedarwood Oil	18	14	3	30	
Fusel Oil	8	14	3	30	
Pine Oil	2	14	3	30	
Origanum Oil (brown)	12	14	3	30	
Creosote	10	14	3	30	
Green Oil	16	14	3	30	
Pyridine	**100**	**14**	3	30	
Dimethylaniline	28	6	3	30	
Carbon Bisulphide	8	6	3	30	
Methyl Salicylate	18	6	3	30	
Butyric Acid	14	6	3	30	
Mono-Brombenzene	**94**	**0**	3	30	
Amyl Alcohol	0	0	3	30	
Acetic Acid	0	0	3	30	
Ethylene Bromide	0	0	3	30	Larvae inert when removed.
Chloral Hydrate	**100**	**0**	3	30	Larvae most inert on removal.
Iodoform	6	0	3	30	
Aniseed Oil	0	0	3	30	
Ethyl Acetate	**100**	**0**	3	30	Larvae all inert on removal.
Iodine	**78**	**0**	—		
Alcohol Absolute	18	0	—		
Ammonia	10	0	—		
Acetone	8	0	—		
Bone Oil	0	38	—		
Carbon Tetra Chloride	68	38	—		
Ammonium Sulphide	0	38	—		
Aldehyde	0	38	—		

The following seemed to be most effective:

Mono-Brombenzene, chloral hydrate, pyridine, ethyl acetate and iodine.

Summary.

In conclusion, the preliminary character of our experiments may again be emphasised. Final conclusions as to the insecticidal value of any preparation can never be drawn, at least with any degree of satisfaction, from laboratory experiments alone. These should obviously be followed by field work under natural conditions, which unfortunately is not possible in this country.

The general results, which we summarise below, are intended therefore to afford some indication as to possible lines upon which future field work might profitably be pursued, rather than as definite recommendations of substances, by the employment of which the Blow-fly Pest may be controlled.

Of substances repellent to the Blow-fly, and therefore capable of protecting sheep from their ravages, the following appear to be the most suitable: methyl salicylate, *p*-nitraniline, picric acid, creosote, green oil, boracic acid, fusel oil, pine oil, alizarine oil, origanum oil, mustard oil, sod oil, iodoform, dimethylaniline, quinoline, allyl alcohol, aloin, saponin, copper carbonate, nitrobenzene, sinapis oil and aniseed oil.

For the application of toxic agents, a powder form has been found to be very convenient and efficient, precipitated chalk forming a suitable and cheap basis. The substances, applied in this form, which appear to be most toxic to the Blow-fly larva, comprise the following: arsenic sulphide, nitrobenzene, eucalyptus oil, methyl salicylate, cedarwood oil, *p*-nitraniline, β-naphthylamine, oxalic acid, borax, quinoline, allyl alcohol, picric acid, dimethylaniline, copper carbonate, oil of cloves, turpentine, β-naphthol, creosote, fusel oil, sinapis oil, aniseed oil and iodoform. Since the young larvae are much more susceptible than the old, in field work, the medicated powder should be applied either previous to, or in the very earliest days of, the larval stage.

Various vapours have been shown to be toxic to the Blow-fly larvae, and of these the most successful are brombenzene, chloral hydrate, ethyl acetate, iodine and pyridine.

ON DISEASES OF PLUM TREES CAUSED BY SOME SPECIES OF *CYTOSPORA*.

By W. N. C. BELGRAVE, B.A.

(*Research Scholar, University College, Nottingham, and formerly Exhibitioner, St John's College, Cambridge.*)

Introduction.

Within the last few years, an increasing number of plum trees in the fruit plantations of Cambridgeshire have exhibited symptoms of a "die-back" disease, followed by the formation of pycnidia, from which red semi-gelatinous tendrils are exuded, these spore-masses belonging to the genus *Cytospora*, the members of which are recognised as conidial stages of various species of *Valsa*.

This investigation was commenced at Cambridge, at the suggestion of Mr F. T. Brooks, to whom I am much indebted for assistance. The work was continued at Nottingham, and I desire to express my thanks to Prof. J. W. Carr for permission to work in his laboratory.

No serious damage to mature plum trees, caused by members of the genus *Valsa*, has hitherto been recorded in England, though Massee (4) has described *Eutypella prunastri* (Sacc.) as attacking young plum stocks. Aderhold (1) in Germany, and Wormald (5) in this country, have described a strikingly similar disease of cherry trees caused by *Valsa* (*Cytospora*) *leucostoma*. As all efforts to obtain mature asci have failed, it is impossible to give the species of the fungus or fungi investigated with any approach to certainty.

Field observations and description of fructifications.

The diseased trees examined belonged to three varieties, Victoria, Prince of Wales, and Pond's Seedling. The trees attacked range from those 4–5 years old, up to the very largest, and the attack usually proves fatal. The first sign of attack is a withering of the leaves, usually progressing from the top of the tree downwards. Next, areas of bark, which may be on the main stem or a side branch, collapse and turn

brown; on these, after a considerable interval, numerous crater-like or lenticular fructifications develop, which almost invariably prove to be pycnidia. A certain amount of gum appears at the junction areas, but considering the proneness of plum trees to gum production its presence cannot be taken as a characteristic of these diseases.

This general description holds for all the trees examined, but there are differences in detail considerable enough to warrant separate treatment. The following record indicates the nature of these.

A. Victoria.

All the specimens, with one exception, were from trees on plantations at Willingham, Cambridgeshire.

(1) A small shoot from an old tree, of which large lateral branches, six years of age, were affected. The shoot was dead. Numerous small crater-like swellings, 1 mm. in diameter, of the bark were in evidence, due to hard pycnidia, seated in the deeper cortical layers. These pycnidia were seated on a poorly developed, dirty-white stroma, sharply limited from the surrounding tissues by a dark tough "skin." Each pycnidium had a single central pore, and was many-chambered: the walls were lined with sparingly-branched hyaline conidiophores, so closely packed as to form a palisade-like tissue.

The spores, borne singly at the tips of conidiophore branches, were continuous, sickle-shaped, hyaline bodies, averaging 7 μ long, and 1·5 μ broad, with two oil bodies. In moist weather, they are extruded in enormous numbers as pink tendrils, semi-gelatinous at first, becoming horny on drying. On wetting, the tendrils disintegrate into their component spores.

(2) A tree about five years old, from the University Farm, Cambridge, was cut down in August, 1913, and kept exposed on the laboratory roof till the following October. It had then developed numerous pycnidia borne several on each erumpent oval dark stroma. As no traces of this fungus were present in August it must here have developed as a saprophyte. Each pycnidium had several pores. Pink tendrils were extruded, made up of spores similar to those described above, but 5 μ long by 1 μ broad.

(3) A tree nine years old, dead. This specimen was taken 18 ins. from the ground line region, which was also the point of attack. The upper part of the tree was still green. The stromata were black, erumpent, and lenticular; each bearing several pycnidia (Figs. 1 and 2). Over the exterior of each fissure was a thin white covering, specially

noticeable on wetting the bark. Dark red tendrils were extruded, spores as above, 5 μ by 1 μ.

(4) A piece of bark only. The stromata were large and pulverulent,

Fig. 2.

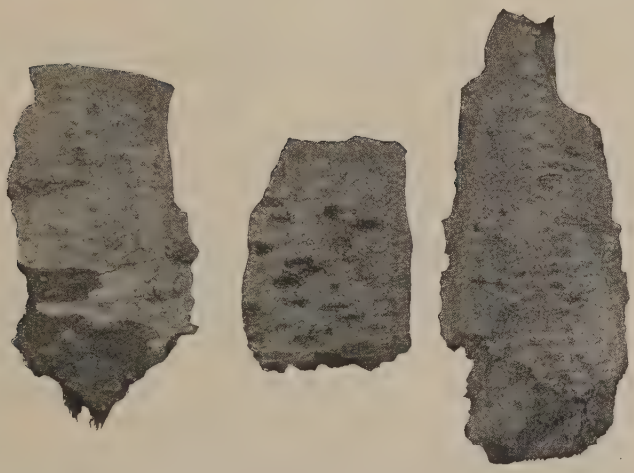

Fig. 1. Fig. 3. Fig. 4.

nearly 5 mm. long, dark olive-green in colour, and sunken in the bark (Fig. 3). There was a sharp line of demarcation from the surrounding tissue. Each stroma bore several immature perithecia.

B. Prince of Wales.

(5) One mature tree was dead, two or three others were dying back from the top. All diseased trees were found at Histon, Cambridgeshire. Small, blue-green, sunken pycnidia were borne singly on poorly-developed stromata, erumpent on keeping (Fig. 4). Pink tendrils of the usual type were developed in a moist chamber.

C. Pond's Seedling.

(6) A mature tree at Long Sutton near Wisbech, one side of which was dying. The disease had spread from above downwards. Pycnidia were borne in groups on sunken, whitish, poorly-developed stromata. There were pink tendrils of spores, the dimensions of which were 5 μ by 1 μ. At the time Mr Brooks obtained this material the foliage of the parts of the tree affected was wilted and brown, presenting a scorched appearance.

(7) Piece of branch about 4 ins. in diameter from a tree at Long Sutton. Pycnidia and spores as in (6) above, but stromata erumpent, and dark-coloured.

It is possible broadly to divide the above into:

(*a*) Those with stromata well developed, dark coloured, and erumpent—Victoria (2) and (3) and Pond's Seedling (7).

(*b*) Those with stromata poorly developed, light coloured, and sunken—Victoria (1) and Pond's Seedling (6).

Too much stress, however, cannot be placed on this classification, in view of the great similarity of the spores and tendrils (except in the case of Victoria (3) where the dark-red tendrils and white crust point to a wider divergence) and also, as will be seen later, on account of the marked influence of media on pycnidial development.

The presence of a stroma more or less deeply seated in the bark, and sharply delimited from the surrounding tissues, indicates that all the fungi belong to the sub-genus *Leucostoma* of the genus *Valsa*, assuming, as is highly probable, that the stroma of the perithecial stage is similar to that bearing pycnidia. Beyond this it is not possible to go. The chief distinction from *Eutypella prunastri* appears to be in the production of well-marked tendrils of spores (cf. Massee, *l.c.*).

Effects of the fungi on the tissues of the host.

The action of the fungi on the tissue of the host has been examined. The stains found most effective in this examination were Delafield's Haematoxylin, and the double stain Picric-Aniline Blue (picric acid being added to saturation).

It is found that the hyphae travel in the soft tissues of the bark, thence spreading laterally into the wood (cf. Fig. 5).

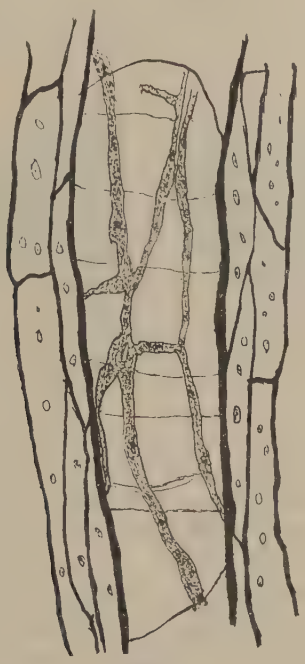

Fig. 5.

In all the tissues gum makes its appearance, in the bark and medullary rays as droplets; in the wood large masses are of frequent occurrence. As pointed out above, the diseased areas are easily identified by their collapsed bark. This collapse is caused by the death and decay of all living cells, only the fibrous cells remaining. It is usual to find above the junction areas of collapsed and healthy areas of bark, considerable lengths of red or brown wood, in which no hyphae can be traced, but gum is abundant (cf. Fig. 6). This phenomenon, however caused, is of common occurrence.

Lateral penetration of the hyphae takes place through the pits which abound in the tissues of the wood (cf. Fig. 7). This penetration is very slow, and often limited to the young wood, *e.g.* in a dead branch 8 ins. in diameter, from a Prince of Wales tree, hyphae could be found in the outermost $\frac{3}{4}$ ins., and in the inner part of this zone only in the medullary rays. In the vessels themselves the hyphae were limited to the outermost $\frac{1}{2}$ inch.

The cell walls are practically unaffected by these fungi, sections from a large dead branch failing to show any discoloration after prolonged treatment with Schultz's reagent; in consequence dead wood, although brittle, shows no signs of crumbling.

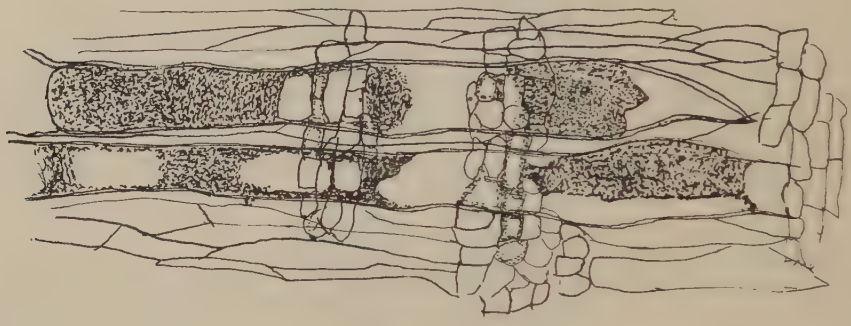

Fig. 6.

The formation of pycnidia is preceded by an aggregation of hyphae in the tissues of the bark to a small hard pustule, which gradually enlarges, splitting the bark in the process. Owing to the friability of diseased bark, this process could not be followed in detail.

In "Victoria (3)," the hyphae are dark coloured, large, fairly thick walled, and stain with difficulty; in the remainder, they are thin walled, narrow, hyaline, and stain readily. With this single exception, the above description applies to all the specimens examined.

Culture experiments and characters of the fungi in pure culture.

All attempts to bring about maturation of the asci in the only perithecium found having failed, the description which follows applies entirely to cultures obtained from conidia.

Separate experiments in regard to the germination of spores were made with all the "strains" mentioned above, and there was complete agreement in the results attained.

Germination readily took place in twenty-four hours at room temperature (summer) in all natural nutriment media, *e.g.* grape juice, fruit extracts stiffened with agar or gelatine, and plum wood extract. Moistened strips of plum wood and solid media, such as carrots or potatoes, also gave good results.

No germination took place in distilled, rain, or tap water, or in water collected after slowly trickling down a healthy plum shoot.

The limits of vitality of the spores are not known, although (*a*) spores readily germinated after five days' soaking in water, when some nutrient material was added, (*b*) spores from tendrils which had been kept for four months in the dry laboratory air readily germinated, (*c*) spores

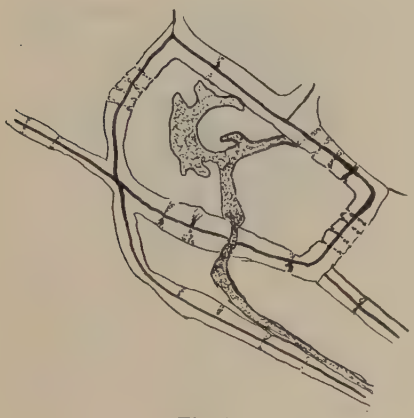

Fig. 7.

Fig. 8.

from tendrils which had been kept three months in culture vessels in a saturated atmosphere, likewise germinated. These facts indicate considerable vitality within wide limits.

Germination tests were also carried out with artificial media. In those containing no nitrogen, such as solutions of glucose, saccharose, or either of these with the addition of phosphates, no germination took place. On the addition of nitrogen in simple combinations, such as ammonium salts, only a slight swelling resulted. Ammonium tartrate and non-poisonous nitrates give better results, and small germ-tubes were produced. The addition of organic nitrogen, *e.g.* peptone or albumen, brought about normal germination.

The details of germination are similar to those described by Aderhold (1). The sickle-shaped spores enlarge considerably, and in about ten

hours, by swelling along the short diameter, are converted into spheres, from which eight to ten hours later 1–4 germ tubes protrude (cf. Fig. 8).

The isolation in pure culture of members of this genus is rendered easy by the production of tendrils of spores. A small piece of tendril is placed in a drop of sterile water, and spores transferred from this to the required medium. When large numbers of cultures were required it was found most convenient to make stock cultures on grape-gelatine, from which mycelial inoculations were carried out.

In nearly all cases, mycelial growth followed by pycnidial formation takes place with great rapidity.

There is an entire absence of any definite stroma, or limiting layer; the pycnidia being developed on small cushions of hyphae (cf. Fig. 9)

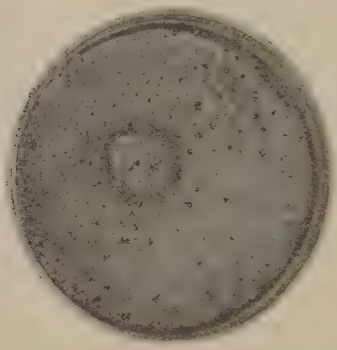

Fig. 9.

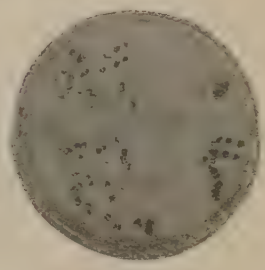

Fig. 10.

and the hyphae of the general tissue passing gradually into conidiophores.

On cutting sections of a very young pustule (it is best to take one growing on agar material), it is seen to be a solid mass of interwoven hyphae. As growth continues, spaces appear, which later become chambers. These chambers are lined with sparingly-branched conidiophores, which are full of minute darkly staining granules, specially noticeable as the conidiophore walls stain only feebly. Later, conidia are abstricted from the tips of the conidiophores. One or more pores now appear in the pycnidia, through which drops of water are extruded, followed by the spores, either as pink tendrils, or more often, owing to the saturated atmosphere, as pink droplets.

The media employed included grape juice, raisin extract, plum wood extract, alone or stiffened with agar or gelatine; artificial nutrient solutions; and solids, *e.g.* potato, carrot, turnip, and plum wood.

Generally, it may be said that liquid media were not favourable to growth; gelatine cultures were marked by rapid and profuse mycelial growth at the expense of pycnidial formation, the pycnidia when formed being rudimentary; on agar, growth was slow, but pycnidia were abundantly formed.

Potato and turnip slices did not prove good media, growth was slow and pycnidial formation scanty. Carrot was highly satisfactory, large tendril-producing pycnidia being rapidly formed.

Growth on wood blocks, in the usual culture tubes, was very rapid on the surface, a dense mycelial felt being produced in five days. Penetration was however slow; in one case, no hyphae were at any depth greater than $\frac{1}{4}$ inch in a block inoculated two months before; after ten months penetration was complete. Pycnidia were formed in greater abundance on those blocks with bark attached; the bark is split by pressure from below, and through the fissures tufts of hyphae come to the surface; on these, pycnidia are developed in 3–4 weeks.

This formation of pycnidia on the surface of the medium, instead of being immersed, has been noted by Aderhold (1), and ascribed to the high moisture content of the air in culture vessels.

All spores produced in artificial culture were found to be of uniform size and shape, 5 μ by 1 μ, hyaline, continuous, and sickle-shaped, thus agreeing with those found in nature.

The following is a detailed description of certain peculiarities in some of the "strains"; for convenience each strain is described by the name and number of its host.

(*a*) *On raisin or grape-gelatine media.*

Victoria (1). Medium rapidly coloured black, hyphae remaining hyaline.

Victoria (2). Mycelium brown, no discoloration in medium.

Victoria (3). Hyphae dark, no discoloration.

Prince of Wales (5). Mycelium confined to the upper surface of medium, forming a light-brown skin. No discoloration of medium.

Pond's Seedling (6). No discoloration of medium. At the edge of the dish a peculiar "efflorescence" of the medium takes place, due to the production of snow-white, feathery aerial hyphae; these sometimes appear in a dish which is drying up.

(*b*) *On raisin, grape juice, or plum agar media.*

Victoria (1). Medium blackened. Pycnidia black, 1–2 mm. diameter covered with a light-grey mycelial felt (cf. Fig. 9).

Victoria (3). No discoloration of medium. Hyphae hyaline at first, dark later. Pycnidia as in (1).

Prince of Wales (5). Colourless mycelium. No discoloration. Pycnidia black, covered with grey felt.

Pond's Seedling (6). White mycelium. No discoloration. Formation of aerial hyphae similar to those produced on gelatine. Large white pycnidia, covered with a greenish felt (Fig. 10).

(*c*) *On potato agar media.*

Victoria (1). Greenish-black coloration of medium. Large pycnidia.

(*d*) *On wheat flour agar media.*

Victoria (1). No discoloration. Cream-coloured, almost invisible, mycelium. Grey-coloured large pycnidia.

(*e*) *On acid and alkaline media.*

By titration with normal acid or alkali, using phenol phthalein as an indicator, a series of raisin agar tubes was obtained, containing various concentrations of acid or alkali. It was found that the degrees of acidity or alkalinity limiting the growth of these fungi were 10 per cent. normal HCl and 5 per cent. normal NaOH.

Victoria (1). 10 per cent. normal HCl. Slow growth. No discoloration of medium.

5 per cent. normal HCl, neutral, and 5 per cent. normal NaOH. Normal growth, with discoloration of medium.

Pond's Seedling (6) and *Prince of Wales* (5). Normal growth at all concentrations, within above limits.

Formation of perithecia.

Prolonged but fruitless attempts were made to induce perithecial formation in culture. These included growing the fungi on pure agar, agar with a high concentration of nutrient material, acid and alkaline media, and upon wood. Cultures were also kept (*a*) at 28° C. for three months, (*b*) frozen, (*c*) exposed throughout the winter on the laboratory roof, (*d*) in the dark; but with negative results.

The recent work of Shear (3) and Harper (2) has indicated the existence of "strains" within the same species of fungus which behave differently in culture media. It seems probable that the fungi here investigated were conidial bearing strains only. The constant cultural differences described above point to the existence of different strains.

Inoculation experiments.

These have, so far, yielded negative results. A T-shaped cut was made in the bark of a healthy plum shoot, and a small piece of mycelium introduced under the edges of the cut.

Inoculations made in September, 1913, were examined a year later but no signs of infection could be found.

In view of the negative results yielded by the infection experiments up to the time of writing conclusive proof that the "die-back" disease under investigation is caused by the fungi in question is lacking. There is however strong presumptive evidence that they are responsible. Their presence in the tissues of the host in each case examined, the general character of the development of their fructifications on the diseased bark, the identity with or close relationship to *Cytospora leucostoma*, a parasitic fungus known to cause a strikingly similar disease on cherry trees, of the fungus isolated from the diseased areas of the affected plum trees in most cases examined, and the failure to find in the diseased parts any other organism to which the disease could be attributed, are points which, taken collectively, suggest that the fungi in question have caused the trouble. The lack of confirmative evidence from the infection experiments, although rendering absolute proof of the cause of the disease at this stage impossible, does not necessarily conflict with the view that these fungi are the cause, since it is always possible that the conditions necessary for successful infection did not obtain in the experiments already conducted.

SUMMARY.

(1) A disease or diseases of plum trees believed to be caused by one or more species of *Cytospora* has been described.

(2) The fungus isolated in most cases is closely related to or identical with *Cytospora leucostoma*.

(3) Complete germination of the spores took place only in presence of organic nitrogen.

(4) Pycnidia and spores were obtained in artificial culture, similar to those occurring in nature.

(5) Attempts to induce perithecial formation failed.

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EXPLANATION OF FIGURES.

Fig. 1. A piece of diseased bark of Victoria (3). Nat. size.
Fig. 2. T.S. of pycnidia of Victoria (3). ×80.
Fig. 3. A piece of diseased bark of Victoria (4). Nat. size.
Fig. 4. A piece of diseased bark of Prince of Wales (5). Nat. size.
Fig. 5. L.S. of diseased wood of Prince of Wales. ×300.
Fig. 6. L.S. of wood of diseased Prince of Wales branch, above hyphae. ×220.
Fig. 7. T.S. of diseased wood of Prince of Wales. ×800.
Fig. 8. Germinating spores of fungus from Victoria (1). ×300.
Fig. 9. Petri dish culture of fungus from Victoria (1) on raisin agar.
Fig. 10. Petri dish culture of fungus from Pond's Seedling (7) on raisin agar.

STUDIES IN ENCHYTRAEID WORMS. *HENLEA FRAGILIS* FRIEND.

By the Rev. HILDERIC FRIEND, F.R.M.S.

(With Plates XXVII—XXXII.)

The object in view in preparing these Studies is to supply an accurate and detailed account of British White Worms or Enchytraeids. These researches have been carried out in the Department of Agricultural Zoology, Birmingham University, which is under the direction of Prof. Gamble, D.Sc., to whom the writer here acknowledges his indebtedness.

In the following pages the Genus *Henlea* is the subject of study, the type chosen being *Henlea fragilis*. The history of the genus is first set forth, and previous definitions examined. These are found to be unsatisfactory, and a new definition is proposed. The history of the type is then given, with a detailed account of the external characters and internal organs. This leads up to the definition of the species, and the discussion of its systematic position, which is shown to be related on the one hand to *Henlea hibernica* Southern, and on the other to the American species *Henlea moderata* Welch.

The group of worms known as Enchytraeids is a large and important one. While the species are, on the whole, known to be of great service in relation to Agriculture, there has for some time been a suspicion that in certain cases they are injurious to plants, and these studies arise out of efforts now being made to determine the question of their value. The family name *Enchytraeidae* is of interest in this connection. By its etymology (*ἐν*, *in* and *χύτρος*, *a flower pot*) we learn that the type was first observed in gardens, and might be regarded pre-eminently as the "pot worm." Later research, however, reveals the fact that the various species and genera included in the family enjoy the widest possible distribution in this country, being found not only in pots and flower borders, but in manure heaps, leaf mould and vegetable refuse of all kinds, from low tide mark on the seashore to the tops of our highest hills.

No fewer than 11 genera of Enchytraeids are now known to occur in Great Britain. Taken alphabetically they stand as follows: *Achaeta*, *Bryodrilus*, *Buchholzia*, *Chamaedrilus*, *Enchytraeus*, *Fridericia*, *Grania*, *Henlea*, *Lumbricillus*, *Marionina* and *Mesenchytraeus*. Of these, *Henlea* is the first in order of treatment by Michaelsen (6), and a typical British species of this genus has been chosen as the first representative of the family for treatment in this series of studies.

The genus *Henlea* was established by Michaelsen (5) in 1889, six species being then recognized. Though the number was so limited, the characters were somewhat heterogeneous. The main features were as follows: Setae not of uniform size or arrangement. Head pore between the prostomium and peristomium or first body ring (usually represented by the symbol 0/1). No dorsal pores. Blood colourless. Nephridia with the duct arising near the septum. Dorsal vessel arising in front of the clitellum. Oesophagus sharply marked off from the intestine.

All the characters here enumerated, however, may be found in one or other of the allied genera. In 1895 Beddard (1) discussed the definition, which was five years later further extended and modified by Michaelsen (6), who at this time admitted only five species as beyond dispute. He drew attention to the following points: Coelomic corpuscles (Lymphkörper, lymphocytes) of one form only, large, mostly discus-shaped, seldom elliptical, darkly granulated. The oesophagus merging suddenly in the intestine in the 7th, 8th or 9th segments. The origin of the dorsal blood vessel anteclitellian in segment 8 or 9, spermathecae lacking diverticula, but communicating with the oesophagus.

In the systematic arrangement the presence or absence of oesophageal glands (Darmtaschen, intestinal diverticula) finds place. The recent discovery of many new species both in Great Britain and abroad, however, shows that not one of the characters in the new definition is strictly generic. We meet with species whose coelomic corpuscles differ from those of the type, others in which the oesophagus does not merge suddenly but gradually into the intestine. In a few instances the spermathecae possess diverticula, and are merely attached to, but do not communicate with, the intestine. Finally, we have what seemed to be the most distinctive characters swept away; for certain species have been discovered whose dorsal vessel does not originate in front of the clitellum, and whose intestine is destitute of special glands (Darmtaschen).

Hence a new definition became necessary. There are many points which would serve the systematist in his endeavours to distinguish

species, though it is very difficult, in the light of the most recent research, to define the genus. The brain, to which no allusion has been made in any of the foregoing diagnoses, is usually of quite a definite type, being as a rule hardly longer than broad, and more or less concave behind. The spermathecae vary; salivary glands (peptonephridia) may be present or absent, as may also the oesophageal glands. The setae may be of the *Fridericia* type, *i.e.* shortest in the middle of each bundle, or of equal length, and the oesophagus may or may not go sharply into the intestine. Sometimes there are glands at the ectal opening of the spermathecae in the intersegmental groove 4/5, at other times they are absent. The coelomic corpuscles may be discoid, as in the assumed type, or irregularly shaped; the salivary glands may or may not open into the intestine, while their position varies greatly. Sometimes they are dorso-lateral, at other times they are ventrally placed, while in a third group one gland is dorsal and the other ventral. Once again the dorsal vessel may arise in segments 7, 8 or 9, in which case we usually find the oesophageal glands near its point of origin, where also the oesophagus suddenly merges in the intestine; or the vessel may originate in or near the clitellum, the oesophageal glands being in this case wanting, while the oesophagus passes gradually into the intestine. When the oesophageal glands are present there may be only one (*Henlea moderata* Welch), or we may find a pair (*Henlea nasuta* Eisen). In at least one instance (*Henlea ventriculosa* D'Ud.) they number four (or two pairs).

Welch has well observed that "Taking the genus as a whole, there is a remarkable variation in the different organs. *Henlea puteana* Vejdovsky is unique in having two pairs of spermathecae. The species of the genus can be grouped in one of several ways according to the criteria, which may be the character of the setae, the presence or absence of intestinal diverticula (Darmtaschen or oesophageal glands), the presence or absence of peptonephridia (salivary glands), the place of origin of the dorsal vessel, or the presence or absence of diverticula[1] on the spermathecae." The nearest allies are *Buchholzia* and *Bryodrilus*. A newly described American species (*Henlea moderata* Welch) with its solitary oesophageal gland brings us very near to these genera, which are also closely approached by other species in other directions.

As a step towards a more satisfactory definition I suggested some time ago (4) that it would be well to form at least two groups, calling

[1] The original reads "ampullae," but what we call "diverticula" are clearly intended.

the species which have no oesophageal glands *Henleanella*, and thus reserving the term *Henlea* exclusively for those species in which oesophageal glands exist. The forms which depart from these two divisions by showing the origin of the dorsal vessel in the region of the clitellum might possibly be arranged in a third group as *Henleana*. In the present paper, however, I do not propose to discuss these forms, but to confine myself purely to those species which, having oesophageal glands on or near the 8th segment, where the dorsal vessel takes its rise, may be regarded as true or typical species.

Henlea thus shorn of doubtful characters would reveal the following points:

1. *Generic.* Glands or diverticula (Darmtaschen or oesophageal glands) arising from the oesophagus in or near segments 7, 8 or 9, and varying in number from one (*H. moderata* Welch) to four (*H. ventriculosa* d'Ud.)[1]. Dorsal vessel anticlitellian, arising immediately behind the oesophageal glands. Oesophagus passing sharply into the intestine where the glands open into it.

Coelomic corpuscles (Lymphkörper) of one form, large, discus-formed or broadly elliptical, darkly granulated. Brain slightly longer than broad, concave or incised before and behind.

2. *General.* Setae straight or slightly curved within, varying both in number and length; often *Fridericia*-like, *i.e.* with the shortest in the middle of the bundle. Small head pore, situated between the prostomium and the first body segment or peristomium, and represented by the sign 0/1. Dorsal pores absent. Blood colourless; vascular system destitute of the so-called heart-body (Herzkörper), though the dorsal vessel is often enlarged in segments 6 to 9.

Nephridia with small anteseptal portion, the postseptal changing into the duct immediately behind the septum. Spermathecae consisting of a sac-like body, usually with a swollen portion or ampulla; glands at the opening in intersegment 4/5 sometimes present, diverticula rare (not yet found in any British species), the posterior portion of the spermatheca attached to the oesophagus or opening into it. Salivary glands present. Three pairs of septal glands in segments 4/5, 5/6 and 6/7. Sperm funnels in the 11th segment with duct ending in the penial bulb on the ventral side of segment 12. Oviduct aborted, the oviducal pore being usually invisible.

It will be seen that one or other of the foregoing general characters

[1] These glands are dorsal to the oesophagus, whereas in Kerria, for example, they are ventral.

is shared by other genera, and that the line of demarcation is anything but sharp and clear. But it must at the same time be noted that if we limit the genus as suggested the oesophageal glands, coelomic corpuscles, and brain are distinct characters separating *Henlea* from every other genus. *Buchholzia* and *Bryodrilus* will then be the only genera at all likely to prove confusing and these can be distinguished by characters which are perfectly definite and satisfactory.

If now we look at one of our indigenous species which approaches most nearly the typical form, and subject it to a very careful and systematic examination, we shall have a standard by which all the other species may be tested. A suitable type presents itself in the case of *Henlea fragilis* Friend, and we chose it partly on account of certain points of interest attaching to its life-history and distribution.

Henlea fragilis was first found by the author at Bopeep, St Leonards-on-Sea, on December 21st, 1911, against the walls of an arch through which a streamlet flows into the sea. Along with it were several other new species, which were described (3) in the *Journal Roy. Micros. Soc.* for 1912 (pp. 586–598). The claims of this species to special recognition lie, not alone in its typical character, or in the fact that I have been able to obtain abundance of living material for microscopic investigation, but also in the following curious coincidence.

In the summer of 1914 a box containing living specimens of Peripatus for research was received at the Birmingham University. As it was found on examination to contain a large number of Enchytraeids, Professor Gamble, F.R.S., kindly handed the material over to me for investigation. The white worms proved to belong to various species and genera, and among them were several finely developed and fully mature specimens of *Henlea fragilis*. The question, how the worms, which had been found by me previously in Sussex, came to be included in earth from Cape Town is one which may be discussed elsewhere. It suffices to say that I was in possession of excellent material both for the study of the living worm and for the preparation of sections, and could therefore produce a fuller and more detailed account of *Henlea* than had hitherto appeared.

In February, 1915, Mr Cox, Steward in the Department of Zoology at the Birmingham University, prepared for me an excellent series of sections both transverse and longitudinal-vertical, and it is upon the study of these sections, carefully checked by a constant reference to the living worm, that the following details are based. The illustrations which accompany the paper have all been made from the microscopical

sections by the aid of Zeiss and Leitz objectives and camera lucida. The worms were kept for a time in damp blotting paper before being killed, in order to clear the intestine and render the making of sections both safe and easy.

1. *External characters of Henlea fragilis.*

An enchytraeid worm with colourless blood, closely related to *Buchholzia* and *Bryodrilus*, of normal size and appearance with an average length of 12–15 mm. and possessing about 50 segments. Each segment save the first and last bears setae (Borsten), but those found on segment 12 disappear as the adult stage is reached and the girdle begins to develop. Apparently the smallest and innermost setae in the ventral bundles of segment 12 are those which hold on most tenaceously, and are the last to disappear: a point which may not be without significance. The setae are arranged in four bundles on each segment (Plate XXVII, fig. 1) and are disposed, not at equal distances around the body, but on the lateral and ventral surfaces. They vary in number in different specimens and at different times. The largest number yet recorded for any bundle is eight, and so high a number has only been found rarely and in perfectly adult forms. There may be as few as two in a set, particularly in immature specimens, and the size varies as well as the number.

The head is nearly oval in longitudinal section, showing a slight but distinct depression in the middle region (Plate XXXII, *dep.*). Between the prostomium and the first body segment the head pore (*h.p.*) is seen. It lies in the intersegment 0/1, but is not easily found in the living worm, though its presence may be readily detected by the stream of coelomic corpuscles which is forced out under the pressure of the cover glass. It communicates directly with the coelom, and gives relief to the body contents. Its close proximity to the brain might suggest that it serves specially to prevent congestion in that region. There are no other pores on the dorsal side.

Vacuolar or glandular cells occur at intervals in the body wall. They are neither so large nor so numerous as those which are found on the girdle, and in neither instance are they arranged in definite rows, as is the case with some of the Enchytraeids. These cells are usually more numerous on the dorsal than on the ventral surface.

The clitellum is exceedingly glandular (Plate XXVII, fig. 2 *A*), the vacuolar cells extending right round the girdle when the adult stage has been perfectly attained. The whole of the 12th segment, with portions

of segments 11 and 13, is taken up by the clitellum or girdle. Underneath the vacuolar or glandular layer is another composed of muscles arranged longitudinally, which extend throughout the entire length of the body. A very thin cuticle covers the outer muscular layer. The anus is terminal, and is usually very glandular.

2. *Internal characters of Henlea fragilis.*

Nervous system. The brain lies in segments 1 and 2, and in longitudinal vertical sections is oval in outline (Plate XXXII, *br.*). Viewed dorsally, however, the brain appears in the living worm somewhat longer than broad (Plate XXIX, fig. 3), nearly or quite straight in front and concave behind. At the same time it must be noted that the posterior margin is liable to considerable modification, and may appear to be straight or even convex in fully adult specimens. It is composed of two kinds of cells, as is also the nerve cord. Two stout strands are given off anteriorly, and these bend down in the front part of the first segment, in the ventral portion of which they combine to form the nerve in segment 2. The structure of the nerve is best seen in transverse sections (Plate XXVII, fig. 1, *n*). The shape of the brain is instructive when compared with that of *Fridericia*, which almost invariably has decidedly convex extremities and is oval in shape as well as in longitudinal section. Beddard has truly remarked that "the form of the brain in these worms is often highly characteristic of the genus or species."

Coelomic corpuscles. These bodies (Lymphkörper or lymphocytes) are very large and conspicuous. Their size in relation to the vacuolar glands of the girdle may be seen by reference to the illustration (Plate XXVII, fig. 2 *B*), while the other figures supply opportunities for comparison with other portions of the system. They agree with those of *Henlea moderata* Welch (8), and are almost round or of a broad elliptical shape. Too much stress must not be laid, as Welch seems to do, on their unequal distribution in the coelom, as they move freely from segment to segment, and though not usually so abundant in the anterior portion of the body they nevertheless have free access to all the front segments, as is shown by the way in which they stream from the head pore when the animal is affected by the pressure of a cover glass on the microscopic slide. The coelom itself calls for no special notice.

Alimentary system. It will be convenient under this head to discuss

a variety of organs, including the pharynx, tongue, oesophagus and oesophageal glands, septal and salivary glands and intestine, in the order in which they occur in longitudinal section. The mouth lies, as usual, on the ventral side of the prostomium (Plate XXXII, *mo.*) with the peristomium or first body segment as a lower lip. Behind the intersegment 1/2 lies a taste organ (Plate XXXII, *t.o.*) or tongue. It arises from the floor of the buccal cavity, and under certain conditions has exactly the appearance of a valve. It projects into the pharynx, and is capable of being moved forwards and backwards. The base of the organ is broad, and the free end pointed. This gives it the outline in longitudinal section of a short curved wedge. A pair of minute processes (not shown in the illustration) may be seen laterally in the posterior region. In view of the fact that the number and arrangement of these organs vary with the species, and may be solitary, paired or even quadrupled, their study is of considerable interest and importance.

The pharynx is situated in the 3rd segment (Plate XXXII, *ph.*) and has a strong dorsal infolding. It agrees in form and structure with that of *Enchytraeus pellucidus* Friend, as described by Stirrup (7). There is no trace of a stylet, neither can I find any evidence of a direct connection between it and the septal glands. The muscles (Plate XXX, *p.m.*) are very strongly developed. Of the septal glands there are three pairs, which, by reason of their staining readily, are conspicuous objects in longitudinal sections. They each consist of two or three unequal lobes (Plate XXX, *s.g.*), the largest of which is dorsally placed, and posterior to the smaller. Their form and appearance may be best judged by the illustration. It will be well to observe that though the septa appear to be wanting in the first four segments, those in 5/6, 6/7 and 7/8 are strongly developed ventrally (Plate XXX, *t.s.*) in order to form a basis or support for the glands, which project forward, and, like the nephridia, each occupy portions of the two segments 4/5, 5/6 and 6/7.

The next organs attached to the alimentary tract which arrest our attention are the peptonephridia or salivary glands. These are developed, one on the dorsal, the other on the ventral surface of the oesophagus (Plate XXXII, *d.s.g.* and *v.s.g.*). Some authors treat them as part of the excretory system, but their use is still questionable, and we therefore prefer to notice them here. They do not appear to open into either the oesophagus or the pharynx, but are apparently blind appendages to the former, extending from the 4th to the 7th segments. The ventral salivary gland is closely attached to the under surface of the oesophagus, and is possessed of a strong outgrowth in segment 4 which

lies in the coelom in near proximity to the nerve cord. It pushes back the septum between the fourth and fifth segments, but does not normally pass into the latter segment with the rest of the organ. Tubules are given off at intervals, and the posterior extremity is branched.

The dorsal salivary gland commences in front of the first septal gland (Plate XXXII, *d.s.g.*) and lies between that body and the oesophagus. It then passes posteriorly along the coelom attached dorsally to the oesophagus, and ends in two or more small branches (Plate XXXII, *b.s.g.*) in the 6th and 7th segments. Though both salivary glands lie in such close proximity to the oesophagus neither has been observed to enter into it. The condition described above is very similar to that which Welch has so clearly set forth in his account of *Henlea moderata*. So far as I am aware these two species, together with *H. urbanensis*, are the only ones in which salivary glands of this type have yet been described. The peptonephridia are certainly very valuable for purposes of diagnosis on account of their great variation in shape, size and position, and will in future play a more important part in both generic and specific description. It should not be overlooked that in this species, nephridia are found in the segments (6 and 7) which contain peptonephridia. (See Beddard I. 47.)

The oesophagus, whose lumen is ciliated, gives rise in segment 8 to a pair of organs known as oesophageal glands or intestinal diverticula (Darmtaschen). These are the chief distinguishing features of the genus, and merit more than a passing study (Plate XXX, *oes.g.*). They are attached to the oesophagus and open directly into it at the point where the latter enters the intestine. They are somewhat heart-shaped, and consist of a number of tubules, arranged irregularly round a central cavity or duct (Plate XXVIII, *cav.*). In *Henlea* as above defined they are always present, but vary in number and position. In *H. moderata* Welch there is only one gland, which tends to relate it to *Buchholzia*, while in *H. ventriculosa* there are four. Usually, however, there are two, and these lie between the 7th and 9th segments. The latest account of these structures is by Welch (8) who complains that most descriptions are very meagre. It may be useful, therefore, if we give some further details of their structure in *H. fragilis*. The glands, though originating in segment 8, are not strictly limited thereto, nearly one-fourth of the organ projecting through the septum into the posterior portion of segment 7. The anterior extremity is more pointed at the front than the section first chosen for illustration (Plate XXX) suggests. The interior tubules are made up entirely of one form of cell, and the

lumen is ciliated. Surrounding the whole is an outer layer which shows no definite structure, but is filled with minute dark-staining points (Plate XXVIII). In this species there are no chloragogen cells as shown by Welch in *H. urbanensis*. The dorsal blood vessel may be seen (Plate XXVIII, *b.v.*) immediately behind the diverticulum, but in front of the septum which it pushes back into the coelomic cavity of the 9th segment.

The intestine commences in segment 9, and is only distinguished from the oesophagus by its greater diameter (Plate XXX, *int.*).

Vascular system. The blood vessels of the Enchytraeids are normally few, and the arrangement is simple. There is a dorsal vessel which arises either in front of the clitellum (preclitellian), within that organ (intraclitellian) or behind it (postclitellian). In all the Henleas as at present defined the dorsal vessel arises immediately behind the oesophageal glands in or near the 8th segment. In *Henlea fragilis* it sometimes appears as if it originated in the anterior portion of segment 9: but sections show that it is wont to push back the septum 8/9 (Plate XXXII, *sep.*) in front of which it commences (Plate XXX, *d.b.v.*). In segments 8, 7, 6 there are enlargements of the vessel, that in 8 (Plate XXXII, *d.b.v.*) being about twice as large as the one in segment 7, which in its turn is of greater dimensions than the one in segment 6. Each vessel contains a substance which coagulates and stains readily (Plates XXVIII and XXXII, *b.v.*). It cannot be seen in the living worm on account of its transparent nature and the absence of a colouring medium. Observations made on sections of a similar character taken from the red-blooded Enchytraeids, however, show that this is the blood-plasm. The dorsal vessel passes forward to the head, giving off three commissures on the way, dips under the brain and bifurcates, so as to form the two anterior branches of the ventral vessel. The disposition is normal and calls for no further description.

Sexual characters. The sexual organs consist of ovaries and testes, together with numerous accessories, such as sperm funnels and ducts, penial bulbs, male and female apertures, and the storing chambers or spermathecae. We begin with the latter as being the first organs which meet the eye when passing from the head backwards.

The spermathecae (Plate XXIX, fig. 1) of *Henlea fragilis*, of which one pair exists, are situated as usual between the first and second septals in the fifth segment, the opening being in the intersegment 4/5, which is destitute of glands. There are no diverticula, but an ampulla is found about mid-way between the two extremities. The posterior

and internal portion of each spermatheca is embedded in the epithelium of the oesophagus (Plate XXIX, fig. 2, *sp.*) but does not open into the digestive tract, as it is said to do in other species. It simply ends blindly in the tissues of the intestinal canal. Neither do the posterior extremities of the two spermathecae join each other as is the case, for example, with those of *H. urbanensis* Welch and *H. moderata* Welch.

The sperm funnels (Plate XXXI, *f.*) whose mouths are usually filled with masses of spermatozoa lie in the 11th segment. They are only slightly longer than broad and are not perfectly symmetrical in shape, the portion lying towards the dorsal side of the body being enlarged. The collar is moderately large and slightly curved, and surrounds the opening to the duct which passes through the septum into segment 12. Here the duct is long and coiled (Plate XXXI, *d.f.*), but, unlike that of *Enchytraeus pellucidus* Friend and some others, is confined to one segment. It ends in the penial bulb (Plate XXXI, *p.b.*); an organ which has been the subject of careful investigation by Eisen and others. The relative size of the penial bulb can be easily judged by its relation to the sperm funnels in the figure. It is small in comparison with that of some other Enchytraeids and belongs to the group which Eisen regards as lumbricillid in type. It is composed of two kinds of cells and the sperm duct passes through it centrally.

The testes originate in the posterior side of septum 10/11, by which means they are placed in the anterior portion of the 11th segment, in close proximity to the sperm funnels. Here may be found in the fully adult worm, enormous masses of spermatozoa (Plate XXXI, *sper.*) and in good sections the entire process of spermatogenesis can be readily traced. Passing from the male organ in the form of intensely minute spheres they rapidly develop and congregate around the mouth of the sperm funnel, through which they pass to the aperture in segment 12.

The ovaries lie in the adjoining segment, being attached to the ventral portion of the septum 11/12. The developing ova are pushed off and lie in the coelom of segment 12, but the oviduct and its pore are aborted, and every attempt to find out how the eggs are deposited in the cocoon has hitherto resulted in failure.

Nephridia are present in segments 6/7 to 10/11, and again behind the girdle. They are of the usual Enchytraeid type with a small portion in front of the septum and a larger portion (postseptal) behind, which contracts to form the duct immediately behind the septum. The duct and postseptal are about of equal length (Plate XXXI, *neph.*).

Chloragogen cells. It is a usual thing for Enchytraeids to possess

certain special cells on the outer surface of the intestine. As a rule these cells commence about the fifth segment, and extend to the posterior extremity of the body, save that it is no unusual thing for their number to be greatly reduced in the region of the girdle. In this respect *Henlea fragilis* shows a decided departure from the rule, and the absence of chloragogen cells from the first twenty to five-and-twenty segments is a marked characteristic. I have not found them in any instance in front of segment 20. We may now sum up the main points.

Definition of Henlea fragilis.

Length about 15 mm. Segments 55 to 60. Setae 4 to 8, the innermost in each bundle usually the shortest. Girdle extending over one-third of segment 11, the whole of the 12th segment and two-thirds of segment 13. Brain slightly longer than broad; spermathecae without glands or diverticula. Three pairs of septal glands; one pair of oesophageals in segment 8, behind which, in the same segment, the dorsal vessel arises. Nephridia with small anteseptal portion; duct immediately behind the septum as long as the postseptal. Sperm funnels only slightly longer than broad, the duct not centrally placed, penial bulb of moderate dimensions of the lumbricillid type. Coelomic corpuscles large, broadly elliptical; chloragogen cells commencing behind the 20th segment. Salivary glands present, one dorsal, the other ventral, attached to the epithelium of the intestine in segments 4 to 7, branched at the ends.

Observations made on a large number of specimens in various stages of development show the following among other points of interest:

(1) The number of setae seems to increase with age.

(2) The ampulla of the spermatheca appears to enlarge as the adult stage is reached.

(3) The brain tends to become convex posteriorly as the worm grows older.

(4) The salivary glands undergo modification as the animal develops.

Systematic position of Henlea fragilis.

It is not an easy matter to assign to this species of *Henlea* its rightful place. We have agreed, however, for the present, to eliminate all species which do not possess oesophageal glands (*Henleanella*), as well as those species (*Henleana*) whose dorsal vessel is of intraclitellar

origin. This reduces the number of genuine Henleas known to be British (3) to 10 species, viz. *attenuata*, *fragilis*, *fridericioides*, *heterotropa*, *hibernica*, *nasuta* (= *leptodera*), *pusilla*, *quadrupla*, *triloba* and *ventriculosa*.

These species vary in relation to the number, shape and arrangement of their setae, the character of their coelomic corpuscles, the presence or absence of glands to the spermathecae at the opening between segments 4 and 5, the number and position of the taste organs or tongues, the salivary glands, the shape and dimensions of the sperm funnels, the nature of the penial bulb, and the point of origin of the dorsal vessel. In *H. ventriculosa* two pairs of oesophageal glands are present, the other nine species having one pair only. *H. fridericioides* possesses glands to the spermathecae, which also sometimes occur in *H. hibernica*; but as a rule they are absent. Diverticula have never yet been found on the spermathecae of British species. In *H. quadrupla* there are four pairs of septal glands while as a rule three pairs only are present. On the whole *H. fragilis* approaches Southern's *H. hibernica* more nearly than any other British species. This may be shown as follows:

	H. fragilis Friend.	*H. hibernica* Southern.
Length	15 mm.	15–20 mm.
Segments	55–60	55–60
Setae	4–8	5–9
Oesophageal glands	One pair in seg. 8	One pair in seg. 8
Dorsal vessel	8/9	8/9
Contractile swellings	6, 7, 8	6, 7, 8

The brains are also of the Henlean type. But while *hibernica* is opaque, *fragilis* is transparent; *hibernica* often has small spermathecal glands which are not found in *fragilis*; the sperm funnels of *hibernica* are three or four times as long as broad, and the pair of salivaries are ventral, whereas in *fragilis* one is ventral and the other dorsal, while the sperm funnels are only slightly longer than broad. If we look particularly at the salivary glands we have to go to America for a case similar to that of *H. fragilis*. While no other true British *Henlea* has yet been found whose salivary glands are so arranged, Welch (8) has recently described species (*H. moderata* and *H. urbanensis*) which show the same feature. But in the former four taste organs are present, there is only one oesophageal gland, and the dorsal vessel arises in the 9th segment, while in the latter there are two taste organs and the dorsal vessel again takes its rise in segment 9. For the present therefore we are content to allow *H. fragilis* to stand related to *H. hibernica* on the one hand and *H. moderata* and *H. urbanensis* on the other.

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EXPLANATION OF PLATES XXVII—XXXII

Plate XXVII, fig. 1. Transverse section through segment 5.
„ „ fig. 2. Girdle glands (*A*) and coelomic corpuscles (*B*).
Plate XXVIII. Oesophageal gland.
Plate XXIX, fig. 1. Diagram of spermatheca.
„ „ fig. 2. Transverse section showing spermathecae ending blindly.
„ „ fig. 3. Dorsal view of brain, in outline.
Plate XXX. Long. vert. section through segments 4–11.
Plate XXXI. Long. vert. section through segments 9–15.
Plate XXXII. Long. vert. section through segments 1–9.

A. Glands in girdle segment. See *v.g.*
B. Coelomic corpuscles to same scale. See *c.c.*
I–XV. Segment numbers.
a.s. ampulla of spermathecae; *br.* brain; *b.s.g.* branches of salivary glands; *b.v.* blood vessel; *cav.* cavity in oesophageal gland; *coel.* coelom; *c.c.* coelomic corpuscles; *cil.* cilia of intestine; *d.* dorsum; *dep.* depression in dorsal portion of head; *d.b.v.* dorsal blood vessel; *d.f.* duct of sperm funnel; *d.s.g.* dorsal salivary gland; *f.* funnel; *g.* girdle; *h.p.* head pore; *int.* intestine; *l.s.s.* lateral setae sac; *m.* muscles; *mo.* mouth; *n.* nerve cord; *neph.* nephridium, *oes.* oesophagus; *oes.g.* oesophageal gland; *p.b.* penial bulb; *ph.* pharynx; *p.m.* pharyngeal musculature; *pr.* prostomium; *sep.* septum; *s.g.* septal glands; *sp.* spermathecae; *sper.* spermatozoa; *t.o.* taste organ; *t.s.* thickened septa to carry glands; *v.* ventral surface; *v.g.* vacuolar glands; *v.s.g.* ventral salivary gland; *v.s.s.* ventral setae sac.

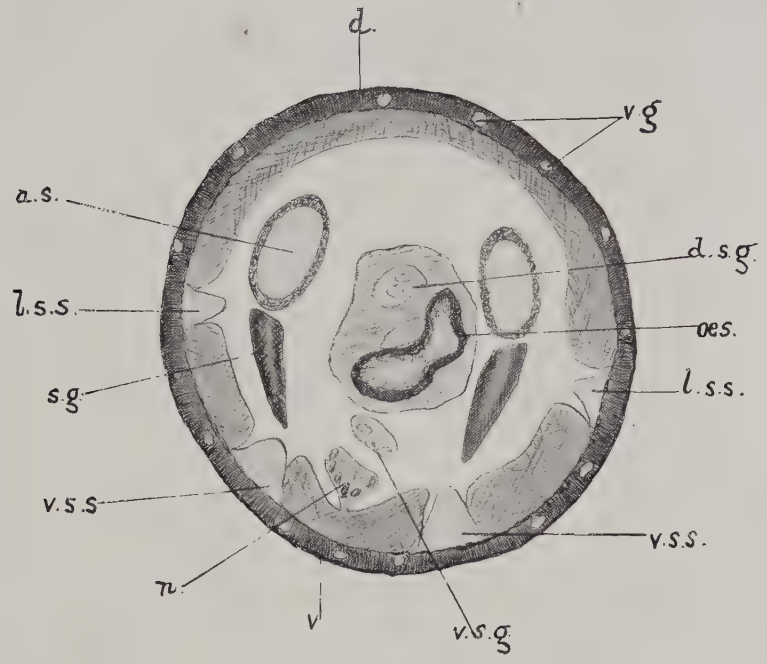


Fig. 1

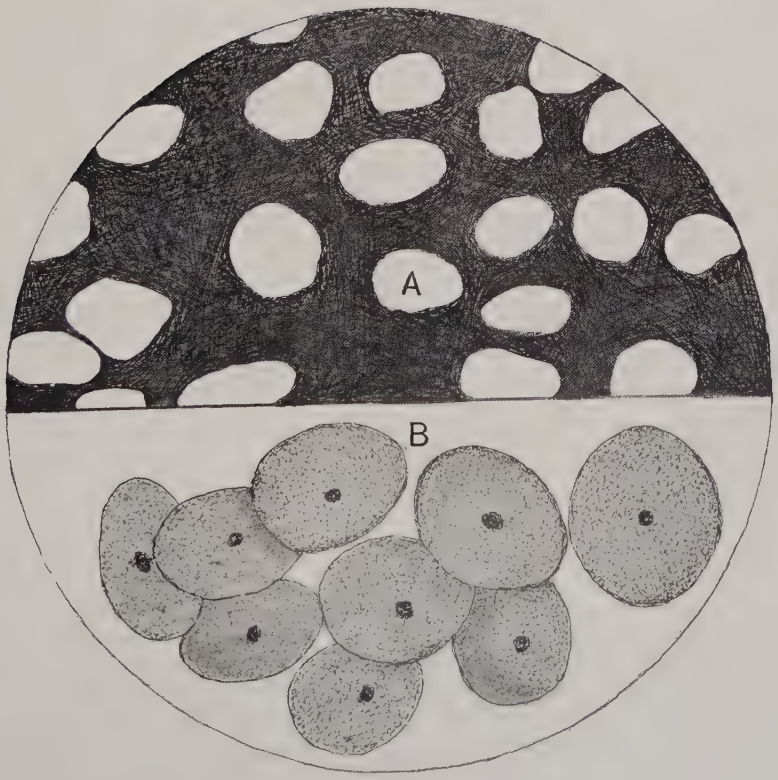
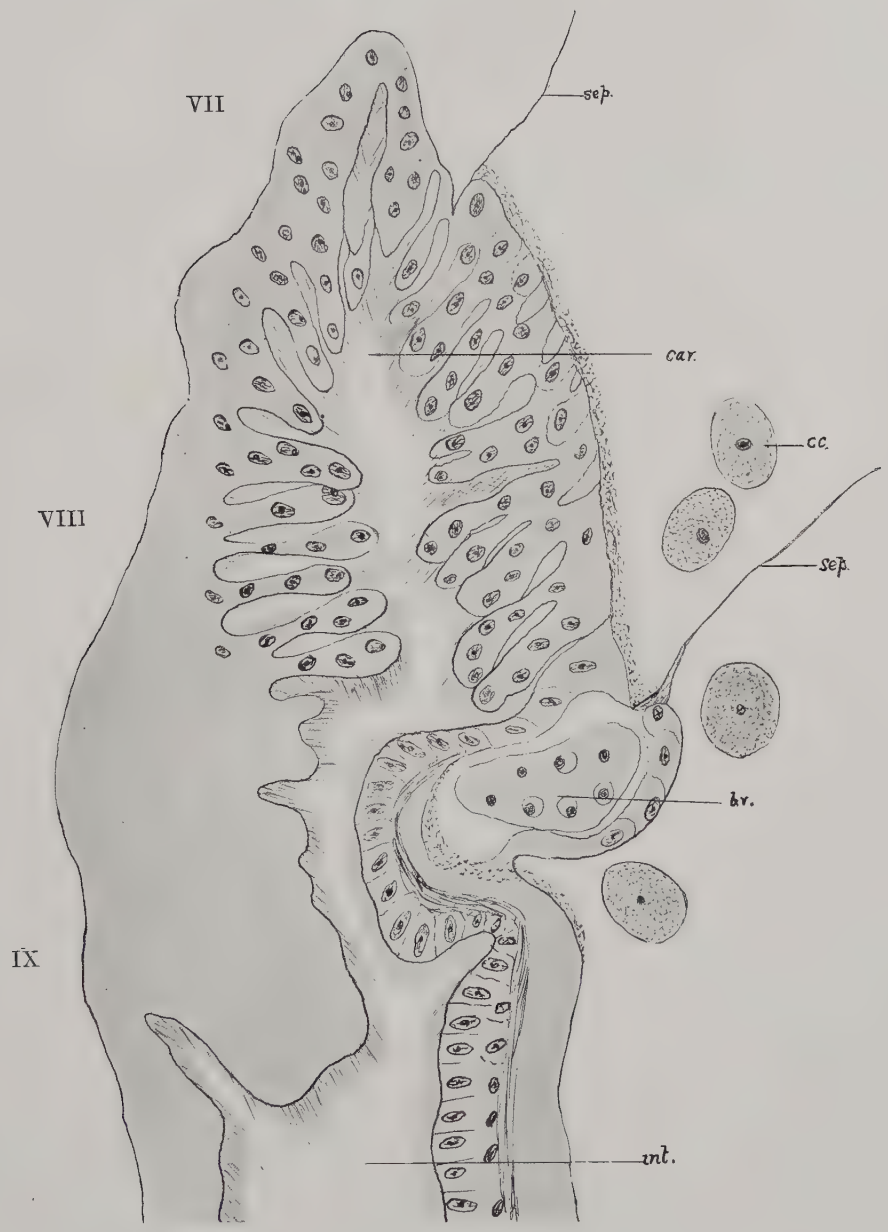


Fig. 2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VIII
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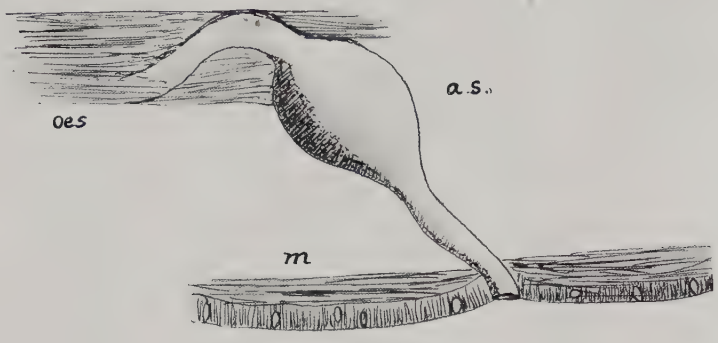


Fig. 1

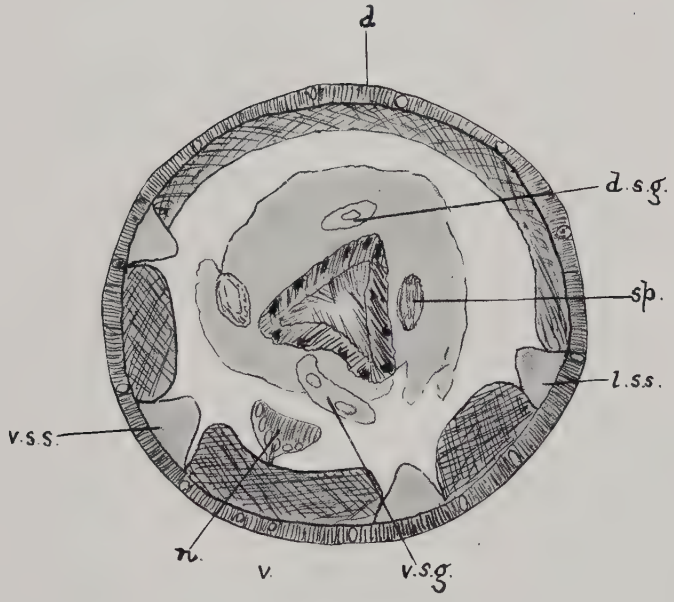


Fig. 2

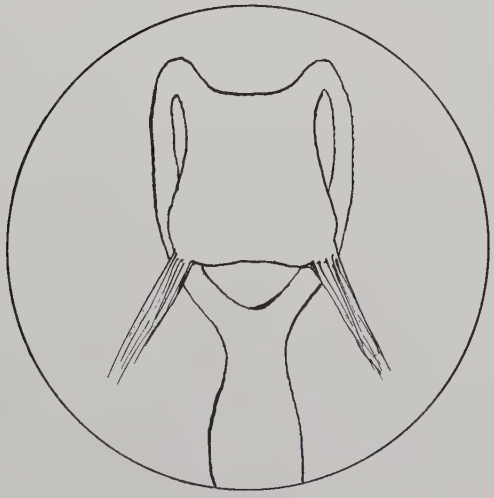
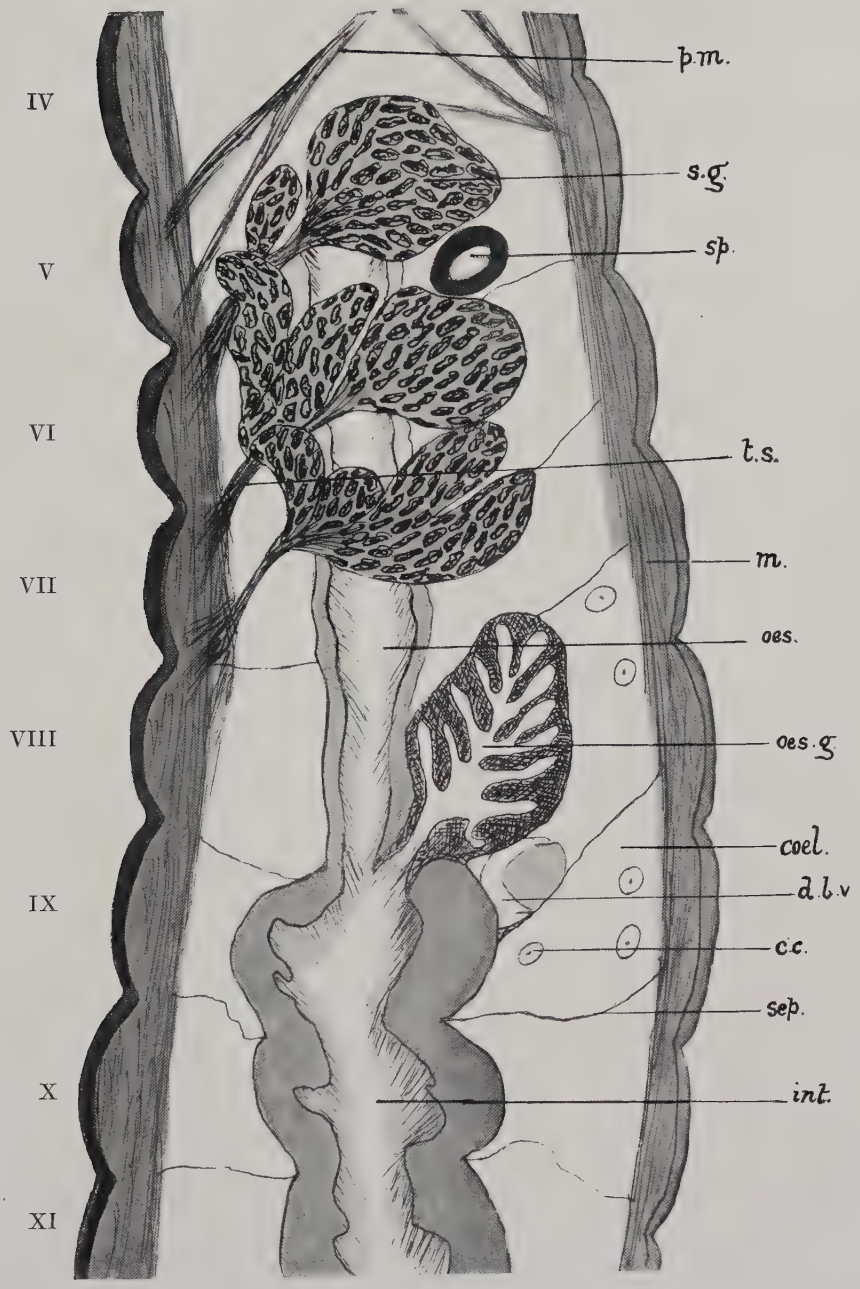


Fig. 3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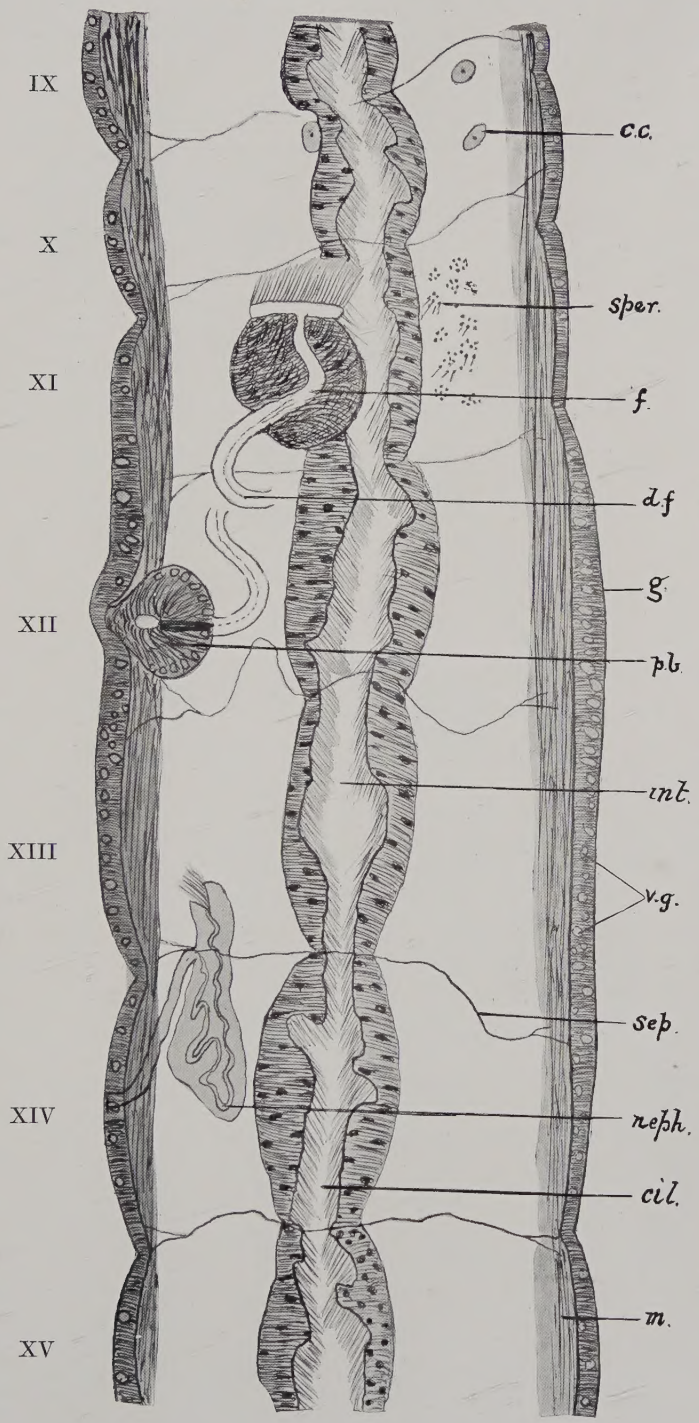
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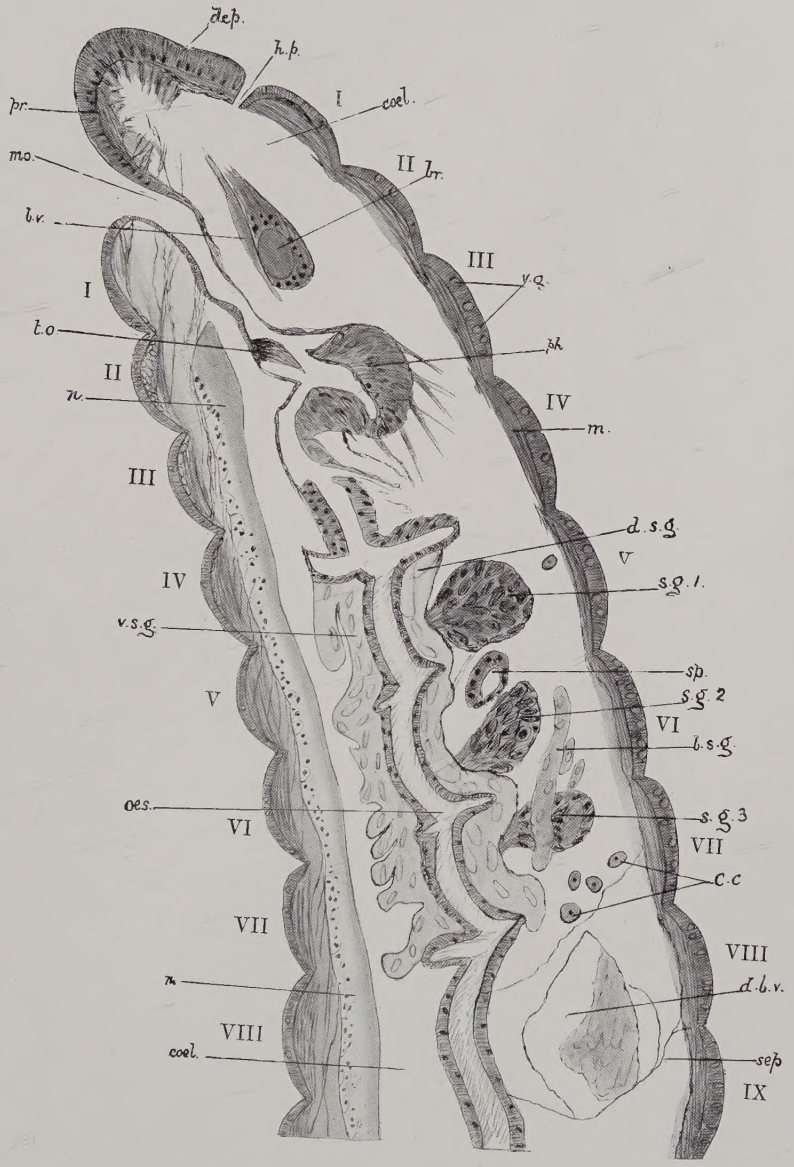
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IX